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This Issue:

Crib-O-Gram
Kwashiorkor in the U.S.
Salt and Pregnancy
Motion Sickness
Interferon Action Studied

by
Gail Shiner Makulowich

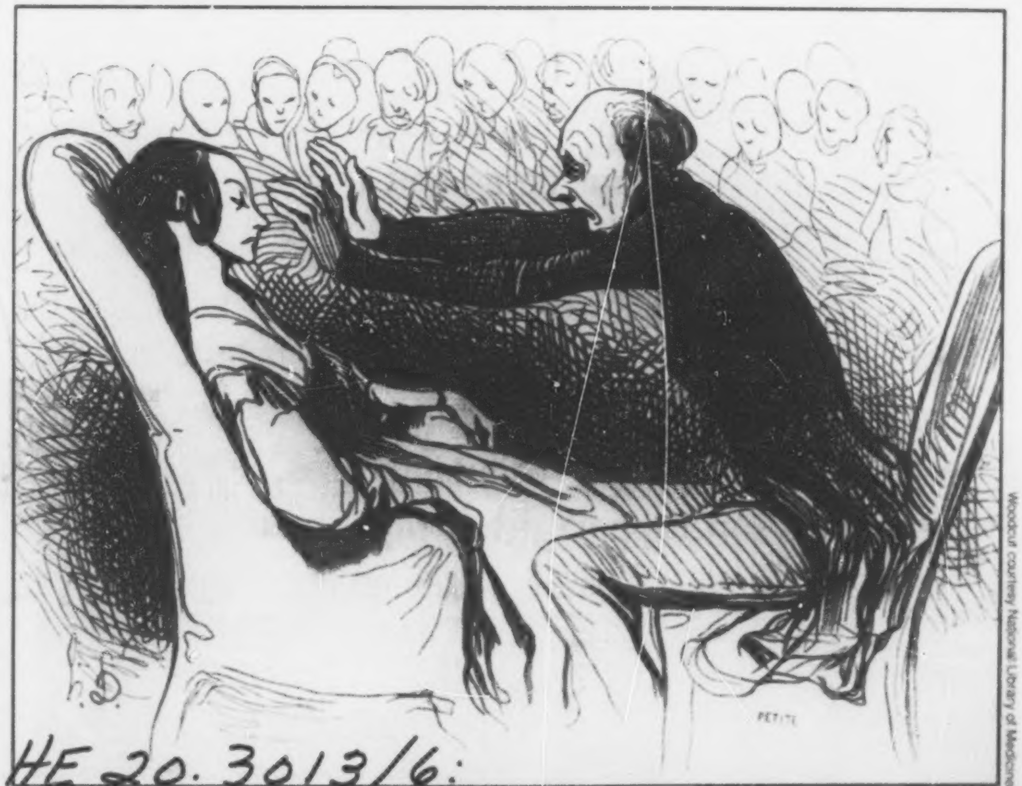
Pilot Research Explores Clinical Possibilities of Hypnosis

Once considered a province of charlatans, hypnosis is now being cautiously examined by clinicians as a treatment to relieve some of the pain, anxiety, and other effects of chronic disease. And self-hypnosis is being considered as a potential tool for chronically ill patients to use to control the pain and anxiety associated with medical crises that occur away from the hospital.

There are numerous reports of single cases demonstrating the effective use of hypnosis to alleviate these problems. However, there is a need for systematic research to determine that the effects seen are directly attributable to hypnosis and not to other factors. Several pilot research projects supported by the Biomedical Research Support Grant Program of the NIH Division of Research Resources have begun to explore what constitutes the hypnotic state, how hypnosis is mediated in the brain, and how it might fit into the clinical treatment of patients with chronic disease.

Hypnosis is described by researchers as an altered state of consciousness characterized by a person's focused attention on potent internal mental images to the exclusion of the external environment. As a person becomes more involved in the

Woodcut from the 18th century of a man "mesmerizing" a young lady: *no longer the province of charlatans, hypnosis is now being seriously considered as a way to relieve some of the pain and anxiety associated with chronic disease.*



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HYPNOSIS *continued*

internal experience of heightened imagery, he enters what is called a trance. "In order to experience hypnosis there must be a sense of immediate involvement in one's mental imagery and detachment from the surrounding external environment," says Dr. Erika Fromm, professor emeritus of psychology at the University of Chicago.

Depending on the images suggested by the hypnotist (heterohypnosis) or by oneself (self-hypnosis), a variety of physiological and emotional states result. Hypnotic suggestions can be made in relation to sensations of taste, touch, smell, and sight, to emotions of happiness and self-confidence, as well as to memory and thoughts. Suggestions can also be made concerning expectations about forthcoming experiences or activities, usually called posthypnotic suggestions. A typical hypnotic suggestion familiar to most laymen is that made by a stage hypnotist to a volunteer subject. He suggests that the pencil he will touch to the hypnotized subject's hand is in fact a lighted match. In response to the suggestion, the subject quickly pulls his hand away from the pencil. In clinical situations, the doctor might suggest to an asthmatic patient the image of his constricted bronchi opening to relieve labored breathing and avoid an asthmatic attack.

Note

The cartoon that appeared on page 14 of the January 1982 *Research Resources Reporter* is the creation and copyrighted material of Mr. Sidney Harris, an award-winning artist whose work appears regularly in several magazines including *Discover* and *Playboy*. The credit line and copyright notice were inadvertently omitted.

There are a variety of techniques used to induce hypnosis, depending on the age and circumstances of the patient and the setting. However, most techniques include: repetition of a monotonous stimulus such as the sound of a metronome or counting breaths in self-hypnosis, or the hypnotist's voice in heterohypnosis; narrowing of the subject's attention, usually through eye-fixation on a particular spot or object; relaxation of body muscles; and reduction or blocking out of environmental stimuli by using a quiet, isolated environment and by focusing attention on a single stimulus.

Research at the University of Chicago laboratory showed that the cognitive and perceptual processes involved in the heightened imagery so characteristic of hypnosis are infused with more intense emotions than waking-state phenomena, according to Dr. Fromm. These images seem especially real to the hypnotized person.

"In self-hypnosis, hypnotic imagery is that which a person conjures up himself," she says. "It can rise to consciousness of its own accord, but it is not likely to be unbidden imagery that overwhelms the subject, as does the imagery of nocturnal anxiety dreams or of psychedelic drug 'trips.' In self-hypnosis, you can suggest to yourself most of the images you wish to come into consciousness."

The impact of hypnotic imagery and suggestions made on a person's emotions and physiology during a trance is a source of puzzlement to researchers, since the hypnotic state has not been characterized by any specific pattern of physiological changes. Nevertheless, some researchers suspect that the striking changes that can be induced by hypnosis and that have been observed in clinical practice must somehow be mediated by changes in the brain.

"The efficacy of hypnosis in alleviating pain (hypnoanalgesia) is well-documented," says Dr. Barbara B. Domangue, assistant professor of psychiatry and human behavior at Thomas Jefferson University. "Since hypnosis isn't magic, we want to know what biochemical mediation is involved in this alteration of pain perception—an area that has been little investigated."

The recent discovery of the endogenous pain reducers, the beta endorphins, has raised the possibility that these "opiates" produced by the body may be involved in the mediation of hypnoanalgesia, according to Dr. Domangue. Epinephrine, norepinephrine, dopamine, and serotonin are other neurotransmitters which also appear to be important to the perception of and reaction to pain.

In a pilot study to test whether levels of these biochemicals change in relation to a reported decrease in pain following hypnosis, Dr. Domangue measured the serum levels of eight patients with arthritic pain at baseline, pretreatment, and posthypnosis. Data on self-reported pain levels and measures of anxiety and depression were also acquired at these times. Patients were hypnotized using standard induction techniques and were given individually tailored images and suggestions directed toward relaxation, comfort, and pain reduction. The measures of pain, anxiety, and depression following hypnosis showed significant decreases.

"Although the reported mean decrease in pain was significant (from 51.4 to 41.1 on a 100-point scale), we weren't controlling for the placebo effect or the experimental demand effect, which arises from the desire of the subject to give the researcher the expected response. These effects may have strengthened the analgesic response," explains Dr. Domangue. "In this preliminary work we are primarily interested in finding out whether the levels of these neurotransmitters change when pain is reduced following hypnosis treatment."

Although seen by Dr. Domangue as tentative, the results of the pilot research suggest that increased levels of beta endorphin, norepinephrine, and epinephrine are associated with posthypnotic pain reduction. She is currently conducting a study of 11 additional patients with arthritic pain to obtain more data.

Dr. Lonnie Zeltzer, pediatrician and director of the division of adolescent medicine at the University of Texas Health Science Center (UTHSC) in San Antonio, has studied the effectiveness of hypnosis for controlling pain and anxiety. In two preliminary studies, Dr. Zeltzer investigated the effect of hypnosis on pain associated with bleeding episodes in hemophiliac children and adolescents and on reduction of the frequency of asthmatic attacks—often associated with stress—in adolescent patients. These pilot projects suggested the effectiveness of hypnosis. However, according to Dr.

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Zeltzer, the study design did not permit assessment of the extent to which behavioral support or the patient's desire to please the researcher accounted for the findings.

"There are many different ways of helping people cope with stress," asserts Dr. Zeltzer. "Hypnosis is only one of these. And while it may be helpful to some people, it may not be for others. I have found clinical situations in which behavioral support can be more effective than hypnosis. For example, I have used nonhypnotic behavioral support techniques to reduce the anxiety of highly stressed children to a level at which they can benefit from hypnotic imagery."

In an ongoing 3-year study of pediatric cancer patients, Dr. Zeltzer is collaborating with Dr. Samuel LeBaron, clinical psychologist in the division of adolescent medicine and a member of the pediatrics faculty at UTHSC San Antonio. They are assessing the effectiveness of hypnosis compared to nonhypnotic behavioral support in reducing pain and anxiety associated with medical procedures, as well as nausea and vomiting associated with chemotherapy. Behavioral intervention, according to Dr. Zeltzer, is used to make medical procedures such as bone marrow aspiration and lumbar puncture tolerable for children who frequently need to repeat these procedures as part of their therapy. Repetitive treatments such as these often create intense anxiety for children; anxious adolescent patients sometimes resist coming to the clinic or, once in the clinic, resist going to the treatment room.

For the current study, young cancer patients are rating the extent of their pain and anxiety immediately before, during, and after bone marrow aspirations or lumbar punctures. Two independent observers also rate the patient's pain and anxiety during this time. After several baseline procedures during which children receive no specific intervention, patients are randomized into an experimental hypnosis group and a nonhypnotized group that receives behavioral support. The therapist, Dr. LeBaron or Dr. Zeltzer, helps the children and adolescents in the experimental group to use hypnotic imagery during their medical procedures. Patients in the nonhypnotized group are desensitized to the procedures through practice sessions, and the therapist helps them during the procedures with relaxation and distraction techniques. Results with 27 patients indicate that procedure-related pain and anxiety were significantly reduced for patients in the hypnosis group but not for patients in the nonhypnotized group.

The parallel studies of the effects of hypnosis compared to nonhypnotic behavioral support to control nausea and vomiting associated with chemotherapy must follow patients longitudinally. At present the data are too preliminary to draw any conclusions.

While Dr. Zeltzer has seen promising results from the clinical application of hypnosis, she cautions against concluding that

Dr. Erika Fromm, professor emeritus of psychology at the University of Chicago, counseling a hypnotized patient: ***the heightened imagery of hypnosis can produce serious consequences when handled incorrectly.***

HYPNOSIS *continued*

because it worked for a few, it should be used clinically across the board. "There are still too many variables in need of investigation before hypnosis can be used routinely by clinicians for pain and anxiety in children," she says. "If one is able to use hypnosis to decrease symptoms, self-hypnosis may also heighten stress."

Although self-hypnosis may not be used intentionally for this purpose, it can occur spontaneously. For example, one patient in Dr. Zeltzer's study described self-induced imagery of "the needle crunching through my bone" immediately prior to several bone marrow aspirations. This vivid visualization often caused him to run out of the treatment room to avoid the procedure.

Dr. Fromm agrees that the potent imagery which characterizes hypnosis—and is one of the factors that distinguishes it from regular waking consciousness—can have untoward consequences when handled incorrectly. This, she says, becomes particularly important when teaching self-hypnosis to a patient. "I will not teach self-hypnosis to anyone who does not have a relatively strong ego," insists Dr. Fromm, a hypnotherapist and researcher. While there is a clinician or hypnotherapist to guide a person in heterohypnosis, one's own "observing ego" serves as the guide in self-hypnosis. For just as a person can self-suggest positive imagery in self-hypnosis, he or she can also conjure up self-destructive imagery that is counterproductive to therapeutic objectives, according to Dr. Fromm.

Dissociation of the observing ego and the experiencing ego occurs more readily in hypnosis than in the waking state, which is why it is effective against pain, according to Dr. Fromm. Other exclusively hypnotic phenomena include perceptual distortions, age regression, posthypnotic suggestions, and amnesia. These psychological characteristics of hypnosis distinguish it from waking-state phenomena, and, according to some researchers, each altered state of consciousness—for example, transcendental meditation—is characterized by specific patterns of thinking, attention, imaging, and memories.

Based on the widely accepted view that hypnosis is an altered state of consciousness, Dr. Fromm's group at the University of Chicago theorizes that self-hypnosis can be qualitatively differentiated from waking-state and heterohypnotic phenomena. Clinical heterohypnosis is no longer characterized by an authoritarian hypnotist issuing commands to an obedient subject. Today's clinical hypnotist gently makes suggestions based on the subject's particular needs, often using images suggested by the subject. Since the current role of the hypnotist is a relatively minor one compared with the subject's hypnotic aptitude, some researchers believe that all types of hypnosis can be conceived as self-hypnosis.

In a pilot experiment to explore the differences between heterohypnosis and self-hypnosis, Dr. Fromm used a standard series of tests to identify easily hypnotiza-

Dr. Lonnie Zeltzer, pediatrician at the University of Texas, San Antonio: ***using hypnosis to reduce anxiety and pain.***

Dr. Barbara Domangue, assistant professor of psychiatry and human behavior at Thomas Jefferson University: ***investigating the neurochemical changes associated with hypnosis.***

ble subjects. Through several heterohypnotic sessions, subjects learned the main induction and deepening techniques. They were then told to use either these or techniques of their own invention for self-directed responses in the absence of a hypnotist. The subjects practiced self-hypnosis 1 hour a day for 4 weeks, keeping a diary of the length of the trance, its depth (how distant from environmental realities), its content, resulting images, types of self-suggestions attempted for inducing and deepening the trance, and subjective impressions of the experience.

Results indicated many similarities as well as some differences between self-hypnotic and heterohypnotic experiences of the subjects. "Two-thirds of the subjects experienced self-hypnosis as a state in which the mind was free to wander and in which they spent a significant amount of time waiting for and attending to whatever rose to consciousness," says Dr. Fromm. "In contrast, their heterohypnotic experience was characterized by steady, focused attention. They permitted few distractions, found it easy to direct attention to relevant tasks, and saw the hypnotist as a facilitator to the process. Thought associations, bodily sensations, and visual imagery all seemed more frequent in self-hypnosis."

The subjects initially experienced anxiety, skepticism, and self-doubts about their ability to hypnotize themselves. After a week, however, they began to feel more at home with the self-hypnotic state, according to Dr. Fromm. Gradually the trance state felt more natural and comfortable, and the ability to enter it quickly and easily increased, as did absorption, fading of awareness of the external environment, and ability to go to greater hypnotic depth with confidence.

Dr. Fromm found that the subjects, without any suggestion from her, chose to explore their internal world of thoughts, fantasies, and memories to solve personal problems and to learn more about who they were. However, most clinical self-hypnosis consists of the self-directed performance of therapist-taught tasks.

For instance, Dr. Fromm teaches self-hypnosis to some patients with emotional problems so that they can control their anxiety between weekly psychotherapy sessions. The therapist-taught task evolves around the image she or the patient suggests. "The specificity of the image is extremely important," asserts Dr. Fromm.

An image that is particularly useful for treating anxiety, according to Dr. Fromm, is that of the patient's hand grasping an audio volume knob. "I show them that when they turn the knob clockwise, it heightens their anxiety," explains Dr. Fromm. "Just as they can do that, they can also turn the knob counterclockwise and reduce their anxiety. The knob is in the patient's hand, which is a symbolic way of expressing that he is in control, and that he doesn't need to be controlled by his emotions. The whole trend in hypnotherapy is to help the patient to see that he has forces within himself that are coping forces, that are integrating forces." R

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Research on the phenomenology of self-hypnosis at the University of Chicago and on the mediation of hypnoanalgesia at Thomas Jefferson University was supported by the Biomedical Research Support Grant Program of the NIH Division of Research Resources (DRR). Hypnosis pilot research by Dr. Zeltzer and colleagues on hemophiliacs and asthmatics at the University of Texas Health Science Center, San Antonio, was also supported by the DRR Biomedical Research Support Grant Program. The research at UTHSC on cancer patients is supported by the National Cancer Institute.

Research Focus

Crib-O-Gram: Simple Hearing Test for the Newborn

Each year in the United States hundreds, perhaps thousands, of infants are born with significant hearing problems. The exact number is unknown because physicians generally do not test newborns for hearing defects. Typically a year or more passes before hearing problems are discovered. This delay in detection may have permanent consequences, according to Dr. Blair Simmons, an ear specialist at the Stanford University Medical Center.

"Most children begin to learn spoken language about 6 months after birth," he says. "They do a lot of listening before they start imitating other people's speech. So, if a child's hearing problems are not discovered until 1 or 2 years of age, the odds are that the child is never going to be a good speaker or even have normal reading skills. There's also a strong possibility of poor social and emotional adjustment."

Since the late sixties, Dr. Simmons has been working on a system that would detect hearing impairment soon after birth. The system, called the Crib-O-Gram, was put on the market last year and is now being used in this country in about 100 nurseries that care for high-risk infants. About 1 in 50 of these infants is born with hearing problems, according to Dr. Simmons.

Diagnoses with the Crib-O-Gram are based on infant movement and respiration before and after 1-second bursts of sound. A microcomputer, stationed at cribside, automatically triggers the bursts of noise, then records and analyzes the movements.

"There's no certain type of movement that identifies a child as having normal hearing," says Dr. Simmons. "Some will be startled; some will wake up and turn over; others will twitch a limb."

Changes in movement and respiration are analyzed by a program composed of relatively simple algorithms. One such rule scores an infant response if the amplitude of the signal from the transponder increases by 50 percent after the noise. Another algorithm identifies a response as positive if the signal amplitude drops by 50 percent. "Of course the machine has to be smart enough to distinguish between significant and random movements," Dr. Simmons says. "It does that through a strictly statistical analysis."

In the 2½ seconds during which movement and respiration are recorded following a noise, the chance that random movement will occur is 1 in 20, according to studies done by Dr. Simmons. "If you run the test a second time, you increase the odds of random movement occurring after both signals to 1 in 400. A third test raises the odds to 1 in 8,000, and so forth," he says. In the 1- to 5-hour total testing period allotted to each infant, noises can be repeated 30 or more times, greatly reducing the chance of a false-negative (normal hearing) report by the microprocessor.

In tests conducted last year at the Stanford Clinical Research Center and other nurseries in the United States, the Crib-O-Gram showed a 14 percent false-positive error and a 3 percent false-negative error. The most significant number is the low false-negative value, which indicates that only 3 of every 100 children born with hearing impairments and tested with the Crib-O-Gram are missed. False positives are of less concern because all children believed to have hearing problems are tested further to confirm the diagnosis.

Following a positive test, the infant's parents are notified and told to watch for symptoms of

Tiny bones of the middle ear called auditory ossicles translate sound into waves of fluid in the canals of the inner ear; *magnified here on the tip of a finger, they are essential to good hearing.*

Photo by Richard A. Bendurf, CHIF, San Francisco impaired hearing. Six months later the parents are contacted again and advised to have their child's hearing retested.

The Crib-O-Gram is not used for retesting. Instead, the child sits between two loudspeakers. Hearing ability is indicated by whether the child turns toward the noises played over the speakers. Children identified as having impaired hearing are fitted with hearing aids. This same test cannot be used on newborns because they do not have sufficient control over heir neck muscles.

The Crib-O-Gram, developed at Stanford University and being manufactured by a California company, is one of several devices intended for use soon after birth. Other systems being developed by manufacturers to detect infant hearing problems require an array of electrodes, recorders, and sophisticated equipment to analyze brain activity triggered by noises. Data collection with these systems requires the constant attention of highly trained personnel. In contrast, the Crib-O-Gram is run entirely by a microcomputer. "All you do is put the transducer under the mattress, place the miniature loudspeakers near the infant's head, plug the machine into the wall, and turn on the switch," Dr. Simmons says.

Although it is significantly less expensive to purchase and operate than other systems, economic considerations currently prevent the Crib-O-Gram from being used outside nurseries that care for high-risk infants. The overall incidence of deafness among healthy newborns is about 1 in 1,000, says Dr. Simmons, an incidence that is believed to be too low to justify routine screening.

In the meantime, most children with hearing problems will be identified by their parents or grandparents. Recently, public service announcements have been aired to acquaint parents with the signs of deafness. Dr. Simmons and many other professionals in hearing detection agree, however, that these announcements have minimal effect. Parents and their doctors usually assume that the baby does not have a hearing problem, says Dr. Simmons. However, it is important to detect a hearing defect early to avoid permanent damage to the social and emotional development of affected children. **R**

Development of the Crib-O-Gram was funded by the National Institute of Neurological and Communicative Disorders and Stroke. The Stanford Clinical Research Center is supported by the General Clinical Research Centers Program of the NIH Division of Research Resources.

Prompt Diagnosis Enhances Kwashiorkor Treatment

Researchers at the General Clini- al Research Center (GCRC) of he University of Colorado's ealth Sciences Center in Denver ave rehabilitated 12 of 14 kwa- hiorkor victims. Kwashiorkor, a evere illness caused by protein alnutrition, typically has a 50 ercent mortality. The illness ccurs most commonly among overty-stricken children in nderdeveloped countries.

"We attribute our success to rompt diagnosis and treatment complications that frequently evelop during nutritional rehabil- ation," says Dr. H. Peter Chase, a ediatrician at the University of olorado's Health Sciences enter.

Because their immune systems e weakened by malnutrition, hildren being treated for kwa- iorkor often die from infections efore they can fully regain their rength. Pneumonia, diarrhea, inary tract infections, seizures, emors, congestive heart failure, d abnormal bleeding may velop during recovery, says Dr. hase: "If the infections are tected and treated as soon as ey develop, the chances of patient recovery are much higher."

Most patients seen at the Colorado GCRC had been examined by other physicians but had been diagnosed for conditions that frequently accompany kwashiorkor, rather than for kwashiorkor itself, says Dr. Chase. "Physicians in affluent countries often overlook the possibility of kwashiorkor as a diagnosis because it is not expected. Doctors should be aware that each year many American infants develop the condition," he says.

Symptoms include stunted growth, sparse, reddish hair, bald spots on the scalp, lesions on the degenerating skin, and edema. The cheeks, hands, abdomen, and feet often swell due to accumulated fluids.

With his associates, Dr. Chase studied nine infants with kwashiorkor and five with marasmic kwashiorkor, a more severe form of the disorder that is caused by a deficiency of both proteins and calories. Patients ranged from 4 to 18 months of age. The children came from different ethnic backgrounds, but all came from the area around Denver.

Laboratory tests conducted at the GCRC showed that blood proteins of the patients were approximately half of normal levels. Many patients were affected with numerous chemical abnormalities including deficiencies in blood albumin, serum urea nitro-

Photo courtesy National Library of Medicine and World Health Organization

Kwashiorkor victim in underdeveloped country: ***Colorado patients with the illness were typically younger and at greater risk of brain damage.***

gen, blood hemoglobin, and serum sodium levels, and in alkaline phosphatase activity. The six patients tested for bone mineralization all were found to have osteoporosis, a condition that weakens bones.

The researchers primarily used a lactose-free milk formula to treat the patients. The formula, which also contained simple amino acids, replenished the body proteins without requiring the intestine to break down complex nutrients, says Dr. Chase. This type of treatment is needed because the intestine of the kwashiorkor patient is depleted of digestive enzymes including lactase. Most milk formulas cannot be used for treatment because they contain the sugar lactose, which requires lactase for digestion.

The lactose-free formula initially was administered in a diluted form and the dosage was gradually increased. Patients were fed intravenously only if they continued to have diarrhea during recovery. The doctors also supplemented the treatment of some patients with iron, magnesium, zinc, and the vitamins A and B.

During the course of the study, one patient died 4 days after his parents removed him from the hospital for treatment by a local folk-healer. Another patient died from gram-negative sepsis, a bacterial infection.

Dr. Chase says that early diagnosis is important to prevent brain damage and even death from malnutrition. Research has shown that brain development is retarded by protein malnutrition early in life. The brain grows to 80 percent of its full size during the first 3 years of life, says Dr. Chase. Infants who have been deprived of dietary proteins often suffer irreversible deficiency in brain development. Proteins also are required to promote overall growth, combat infections, repair tissue damage, and to regulate respiration, digestion, circulation, and secretion. Infants require significantly more protein per unit of body weight each day than do adults, says Dr. Chase.

"Prevention should be aimed at stressing to parents the importance of infant nutritional needs," he says. "Our patients developed kwashiorkor because parents were not knowledgeable. Kwashiorkor in underdeveloped countries typically occurs because parents cannot afford to buy protein-rich foods. But in our patients, poverty reduced the availability of food only when family funds were spent to support alcoholic parents or when family strife was involved."

The parents of three patients imposed their vegetarian diets on the children without realizing the risks of a protein-limited diet for infants. One patient from a commune of macrobiotic vegetarians whose diet excludes meat, cheese, and eggs refused to drink soy milk, his primary source of nutrition. After several months of the low-protein diet, he lost weight, stopped growing, and developed kwashiorkor. Another patient from a vegetarian family received only wheat-based foods, such as breads and cereal products. A third patient had been weaned only on fruits, vegetables, and water.

In other cases, kwashiorkor was the result of excessively overdiluted milk formulas, either prepared in error or because the formula had been inappropriately continued after intestinal illness, says Dr. Chase. "Many parents withdraw or overdilute milk to treat diarrhea, which frequently precedes kwashiorkor. Kwashiorkor then may develop from the metabolic loss of nitrogen. Most of our patients ate foods extremely high in sugar and starches to compensate for the reduced intake of milk. These foods may provide sufficient calories but not enough protein to keep the body functioning at a normal level."

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Research on kwashiorkor was supported by the National Institute of Child Health and Human Development and the General Clinical Research Centers Program of the NIH Division of Research Resources.

Salt During Pregnancy: Risks For the Unborn

Physicians frequently advise pregnant women to limit salt intake, because excess salt increases blood pressure. Studies with animals at Tufts University School of Medicine suggest that limiting salt intake may also help protect against hypertension in the offspring.

"Much research with both humans and animals has implicated strong genetic as well as environmental factors in the origin of hypertension," says Dr. Eunice I. Bloomquist, a physiologist at Tufts. Together with Dr. Vivian Karr-Dullien, she has carried out studies on the effects of environmental factors, such as a high-salt diet, in animals with a genetic predisposition to hypertension. As a predoctoral student, Dr. Karr-Dullien initiated a series of experiments to determine if high salt in the diet of pregnant rats might contribute to higher blood pressure in the offspring.

Three strains were used in their investigation: the spontaneously hypertensive rat (SHR), genetically disposed to hypertension; the Wistar-Kyoto (WKY), closely related to SHR but having normal blood pressure; and the Sprague-Dawley, a common laboratory rat. Females received either a high-salt diet of 8 percent by weight or a standard salt diet of 0.4 percent by weight for the last 2 weeks of their 3-week gestation periods.

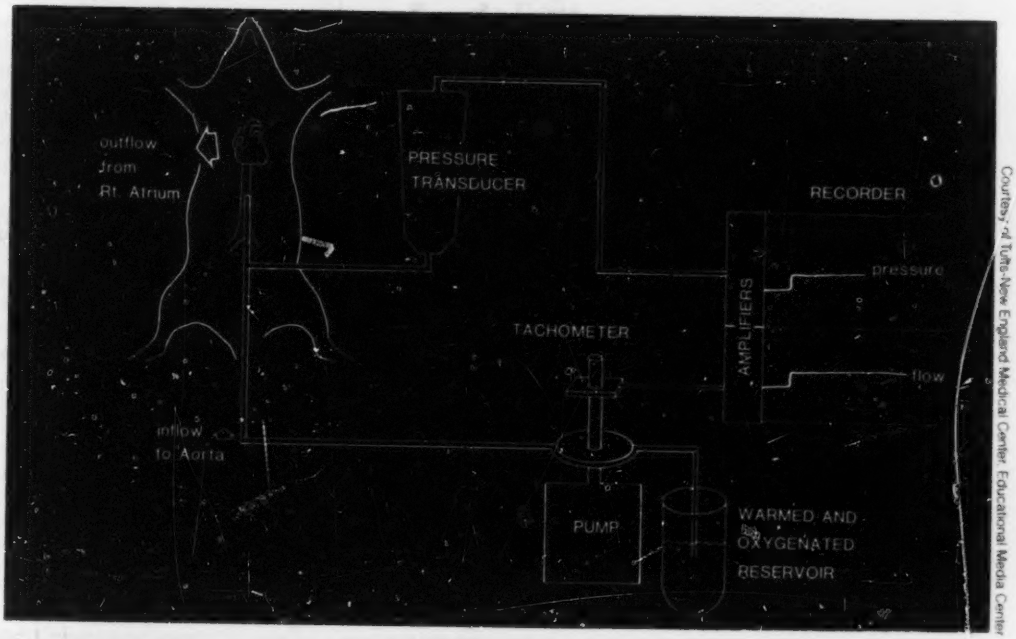
"The most striking finding of these studies," Dr. Bloomquist says, "is that the combination prenatal/postnatal high-salt diet greatly aggravated hypertension in the SHR offspring. These animals developed systolic blood pressures as high as 270 mm Hg, correlated with 63 percent mortality by 4 months of age. There was also high morbidity including stroke and peripheral hemorrhage in many of the SHR offspring. No other animals, including the SHR receiving high salt only postnatally, demonstrated any of these complications. The high-salt diet had no effect on the lifespan of the mothers."

Although the high-salt diet produced little change in the blood pressure of SHR mothers that gave birth successfully, it did increase the rate of unsuccessful pregnancies and prevented maternal weight gain. To distinguish prenatal from postnatal effects of high salt, pups of half the mothers in each strain were switched at birth to lactating mothers on the opposite diet and continued on this diet while weaning. The remaining pups were continued on the same diets their mothers received during pregnancy.

Increased resistance to peripheral blood flow is the underlying cause of many forms of hypertension. This resistance is generally attributed to two structural abnormalities in the arterioles, the small arteries in which most of the resistance to flow occurs. The first of these abnormalities is a thickening of the muscular wall of individual arterioles; the second is a decrease in the total number of arterioles. Whether these structural abnormalities occur before or after the development of high blood pressure—and what type of causal relationship may be involved—has been the subject of much debate in recent years, Dr. Bloomquist says.

To investigate these questions more extensively, she and Dr. Karr-Dullien have developed techniques to simultaneously measure blood pressure and flow in very small animals such as a newborn rat.

Under anesthesia, the aorta of a rat only a few hours old is exposed and a solution containing the plasma expander dextran is pumped into the aorta. The venous outflow takes place through an incision in the right



Newborn rat in hypertension experiment: *measuring blood pressure and blood flow.*

Courtesy of Tufts-New England Medical Center Educational Media Center

atrium. The pump is controlled by a specially designed system which allows for instantaneous, predetermined changes in flow rate. Pressure is measured through a transducer, and flow is measured by a tachometer on the pump shaft. Both pressure and flow are displayed simultaneously on a chart recorder.

Using this system, the investigators have determined aortic pressures at low, intermediate, and high flow rates in newborn and 2-week-old SHR and WKY rats. Contrary to what is seen in he adult SHR, which demonstrate abnormally high pressures over a wide range of flow rates, the newborn SHR demonstrated abnormally *low* pressures as flow rates ncreased. The peripheral resistance at high flow rates was also significantly lower in SHR newborns.

"These findings indicate that he arterioles of newborn SHR exhibit an abnormal ability to expand or distend, compared with hose of the newborn WKY," Dr. Bloomquist says. "After 2 weeks of standard salt diet, both strains vere roughly equivalent in their low-pressure characteristics, but he high-salt diet caused strikingly ower pressures and lower resistance at this age in the SHR."

Other investigators had shown hat arterial smooth muscle from he SHR is generally weaker than hat of the WKY. Dr. Bloomquist is urrently trying to determine: vhether greater distensibility elates to this smooth muscle weakness; whether the higher distensibility is a cause of structural changes such as arteriolar wall thickening and decrease in arterioles, and the specific influence of a high-salt diet on the developing arterioles.

"Our working hypothesis thus far relates to these three questions," she says. "Greater distensibility of the vessel wall in these young animals may lead to abnormally high stress on each vascular smooth muscle cell. The cells would respond to this greater stress by proliferating, so there would be an increase in wall thickness and compensation for the initial weakness. If a high-salt diet aggravated the initial weakness, one could expect greater compensatory responses. It is also possible that greater distensibility in the arterioles might lead to abnormally high tissue blood flow, which is a stimulus for a decrease in the number of arterioles in the mature animal. We are currently studying all of these possibilities."

For further reading see:

1. Karr-Dullien, V., and Bloomquist, E. I., The influence of prenatal salt on the development of hypertension by spontaneously hypertensive rats (SHR). *Proceedings of the Society for Experimental Biology and Medicine* 160:421-425, 1979.
2. Karr-Dullien, V., Bloomquist, E. I., Beringer, T., and El-Bermani, Al-W. I., Flow pressure relationships in newborn and infant spontaneously hypertensive rats. *Blood Vessels* 18:245-252, 1981.
3. Karr-Dullien, V., Bloomquist, E. I., Beringer, T., and El-Bermani, Al-W. I., Arterial morphometry in neonatal and infant spontaneously hypertensive rats. *Blood Vessels* 18:253-262, 1981.

Research into salt, hypertension, and pregnancy is supported by the National Heart, Lung, and Blood Institute and by the Biomedical Research Support Grant Program of the NIH Division of Research Resources.

Squirrel Monkey Model of Motion Sickness

Despite years of study and com- non usage, drugs that counteract he malaise, nausea, and vomiting ssociated with motion sickness re still poorly understood. They re only partially effective and nany must be taken every few ours. The necessity of repeated dosage, with the potential for toxicity and side effects, makes these drugs inadequate, according to Dr. Kenneth Brizzee, a neurobiologist at the Delta Regional Primate Research Center. Designing better drugs to prevent motion sickness is of particular interest to the National Aeronautics and Space Administration (NASA), which looks ahead to continued use of the space shuttle. It is also of interest to the U.S. Navy, which must maintain the health of sailors on long voyages.

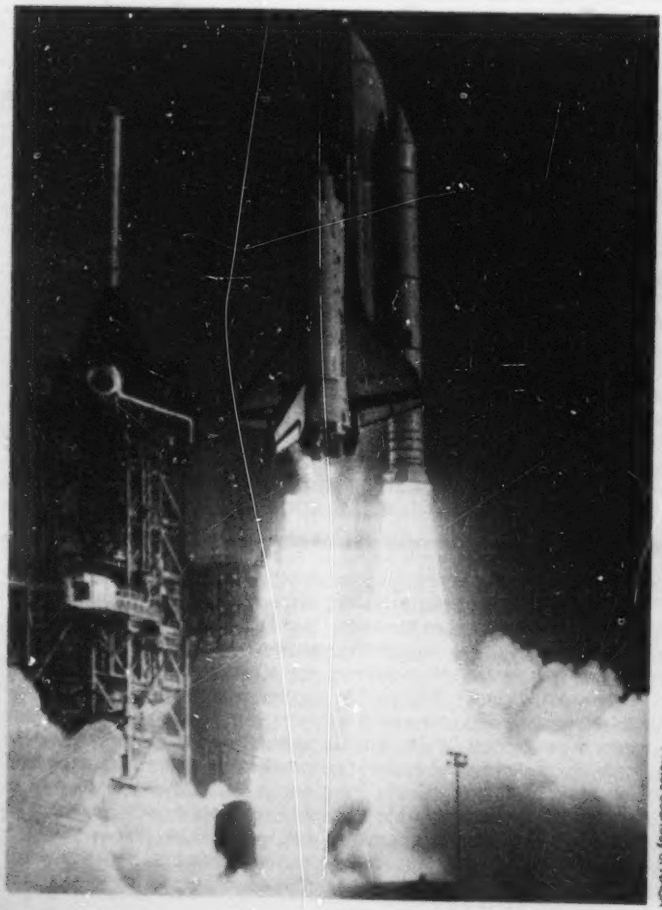
Developing improved drugs to combat motion sickness depends on elucidating the mediating mechanisms of motion sickness in the brain, says Dr. Brizzee. He and his colleagues are using a method developed by Dr. Louis Sokoloff, chief of the Laboratory of Cerebral Metabolism of the National Institute of Mental Health, to map the brain sites involved in motion sickness experienced by squirrel monkeys. The method uses radioactively tagged deoxyglucose to monitor brain activity. Glucose supplies energy to the brain through a series of metabolic cycles. Deoxyglucose forms when an oxygen atom is removed from glucose. The dismembered glucose molecule cannot be entirely metabolized and it localizes in brain tissue. When tagged with carbon-14 (^{14}C) or tritium (^{3}H), it can be traced during a motion regimen to pinpoint areas of the brain involved in, or activated by, motion stimuli.

In their current study, the investigators inject radiolabeled deoxyglucose intravenously into squirrel monkeys selected for their sensitivity to motion sickness. Then the monkey's vestibular system is activated by subjecting it to 1 hour of 25-rpm, rotational-vertical motion. The rotation rate is considered the optimum speed for inducing motion sickness in this species. It was arrived at empirically, according to Dr. Brizzee. He chose the vertical-rotational motion because it activates vestibular receptor cells in all parts of the vestibular labyrinth and, in some respects, is similar to the motion created in the space shuttle.

"Input from the vestibular receptors, which are located in the utricle, saccule, and semicircular canals of the inner ear, from the visual receptors, and less so from the proprioceptors, which are located mainly in the muscles, joints, and skin, provides the brain with data about the relative position of body parts and, thus, one's orientation in space," explains Dr. Brizzee. "It is generally agreed that the primary receptors for motion are located in the hair cells of the vestibular labyrinth of the inner ear. If these receptors are destroyed, there is no motion sickness and it cannot be engendered. A major goal of our research is to identify and locate the structures in the brain stem and cerebellum that are involved in motion sickness and to plot out the circuits or pathways connecting them."

By tracing labeled deoxyglucose in animals experiencing motion sickness, the researchers hope to observe the participating structures all the way from the sensory receptors of the inner ear down through the vestibular nuclei to the structures in the lower brain stem which activate the reticular vomiting center, Dr. Brizzee explains. Activated structures are identified by ^{14}C radioautography, a technique that permits localization of these areas by X-ray films. Black areas in the developed film indicate the location of the radiolabeled glucose in the brain structures, thus outlining the specific sections activated by motion stimuli. Dr. Brizzee is also using ^{3}H radioautography that may enable localization of radiolabeled glucose at the cellular level by exposing frozen photographic emulsion contacted to frozen tissue sections. Micropunch samples—tiny brain plugs—from structures or areas suspected of being involved are placed in a scintillation counter to quantify their radioactivity.

"In this way we're attempting not only to identify the involved structures, but also to quantify the radioactivity in these structures all through the brain stem and cerebellum," explains Dr. Brizzee. Based on present data, the structures that appear to be integral components of the motion sick-



Photos courtesy of NASA

ness emetic circuits are the area postrema and certain adjacent structures, the vestibular nuclei, certain cerebellar folia and nuclei, and the reticular vomiting center. The data indicate that the medial vestibular nucleus is very active at the pontine and upper medullary levels but less so at the lower medullary level of the brain stem.

"We haven't looked at the cerebellum very carefully yet," says Dr. Brizzee. "When we do, we're quite sure that we'll see some interaction between the medial vestibular and cerebellar structures. Once these structures are conclusively identified, we'll use the electron microscope to discern what types of synaptic connections exist."

Identifying the chemical transmitters at these synaptic sites is an additional goal of Dr. Brizzee. Recent studies have suggested the possibility that a neurotransmitter imbalance may be responsible for motion sickness susceptibility and severity. "This is a cautious guess," says Dr. Brizzee. "We'd like to know what systems of neurotransmitters are involved in vomiting induced by motion sickness. When such information is available we may be able to develop antagonists to these transmitters that will prove more effective than the antiemetic drugs now being used."

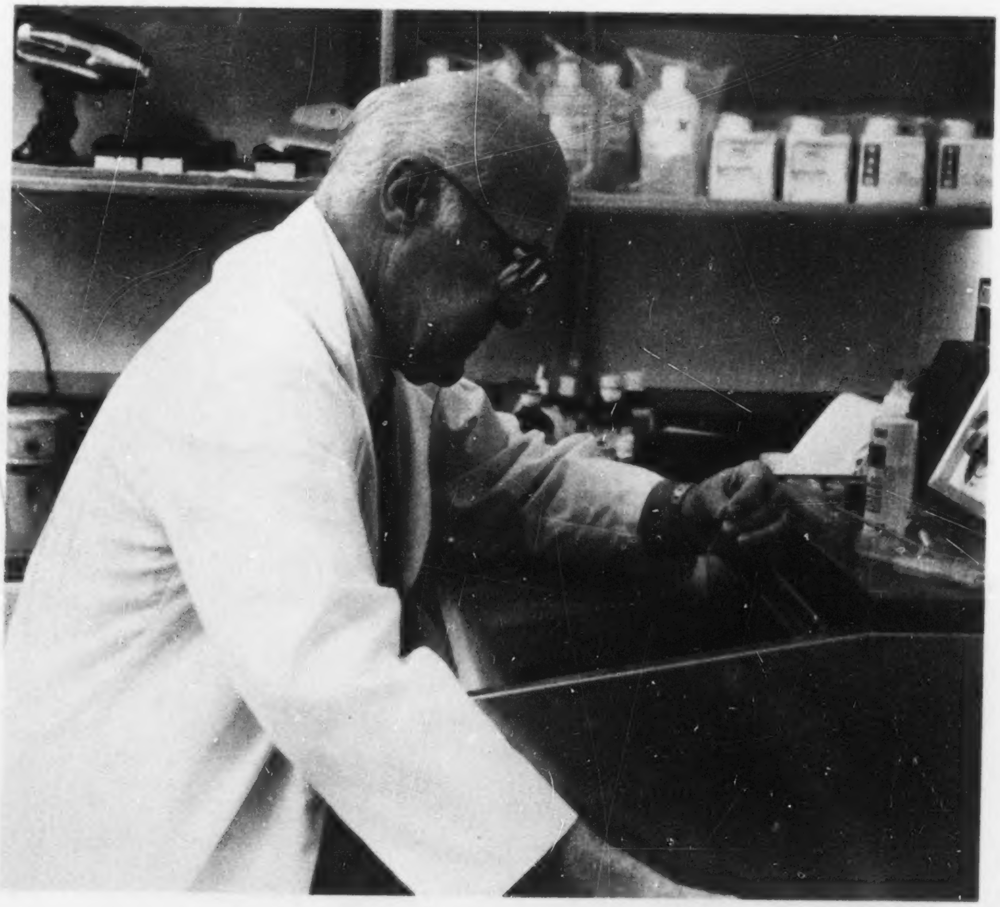
To better understand the action of currently used antiemetic drugs such as Dramamine, prochlorperazine, and amphetamine-scopolamine, Dr. Brizzee is planning parallel research at the primate center to tag antiemetic drugs radioactively and determine their sites of accumulation in brain structures. He is also planning to collaborate with investigators at the Louisiana State University School of Veterinary Medicine to use cineradiography (motion X-ray films) to study sequential internal events in the gastrointestinal tract and body wall muscles during vomiting induced by motion sickness.

For additional reading see:

1. Ordy, J. M., and Brizzee, K. R., Motion sickness in the squirrel monkey. *Aviation, Space and Environmental Medicine* 51(3):215-223, 1980.
2. Brizzee, K. R., Ordy, J. M., and Mehler, W. R., Effect of ablation of area postrema on frequency and latency of motion sickness-induced emesis in the squirrel monkey. *Physiology and Behavior* 24:849-853, 1980.
3. Schneider, R. C., and Crosby, E. C., Motion sickness: part I—a theory. *Aviation, Space, and Environmental Medicine* 51(1):61-64, 1980.

Research on motion sickness in the squirrel monkey is supported by the Animal Resources Program of the NIH Division of Research Resources and by the National Aeronautics and Space Administration.

Launch of the first space shuttle, its pilot, Navy Captain Robert L. Crippen, in state of weightlessness encountered in space flight: ***as the shuttle program accelerates, NASA is searching for better drugs to combat motion sickness.***



Dr. Kenneth Brizzee working at the cryostat: ***mapping brain sites involved with motion sickness in susceptible squirrel monkeys.***

Information Exchange

Research Highlights

A possible target for interferon action in the mammalian cell was recently discovered by researchers at New Mexico State University (NMSU) at Las Cruces.

The suspected target is an enzyme called ornithine decarboxylase (ODC). Interferon rapidly inactivates this enzyme, apparently indirectly by phosphorylation. Phosphorylated ODC may serve as a marker of interferon action.

"The appearance of this phosphorylated enzyme appears to be one of the most apparent differences between interferon-treated and control cells," says Dr. Glenn D. Kuehn, professor of chemistry at NMSU and leader of the research team. "It has appeared only under conditions in which the antiviral state is induced."

The inactivation of ODC by interferon seems to be especially significant since ODC activity is greatly stimulated by tumor-promoting agents and most hormones and in the transformation of normal cells to cancer cells by certain viruses. Cells stimulated to proliferate have shown a rapid increase in ODC activity.

Just recently Drs. Kuehn and Valerie Atmar, in collaboration with Dr. Vaithilingam Sekar of the Memorial Sloan-Kettering Institute for Cancer Research, found that a polyamine-activated protein kinase, which is induced by interferon, inhibits ODC by phosphorylation in mouse ascites tumor cell cultures. Dr. Kuehn believes that the enzyme induced by interferon may be ODC kinase, an enzyme that he and his coworkers recently discovered in the nucleus of the slime mold *Physarum polycephalum* and in rat liver cells. In the presence of certain polyamines, ODC kinase phosphorylates ODC and inactivates the enzyme.

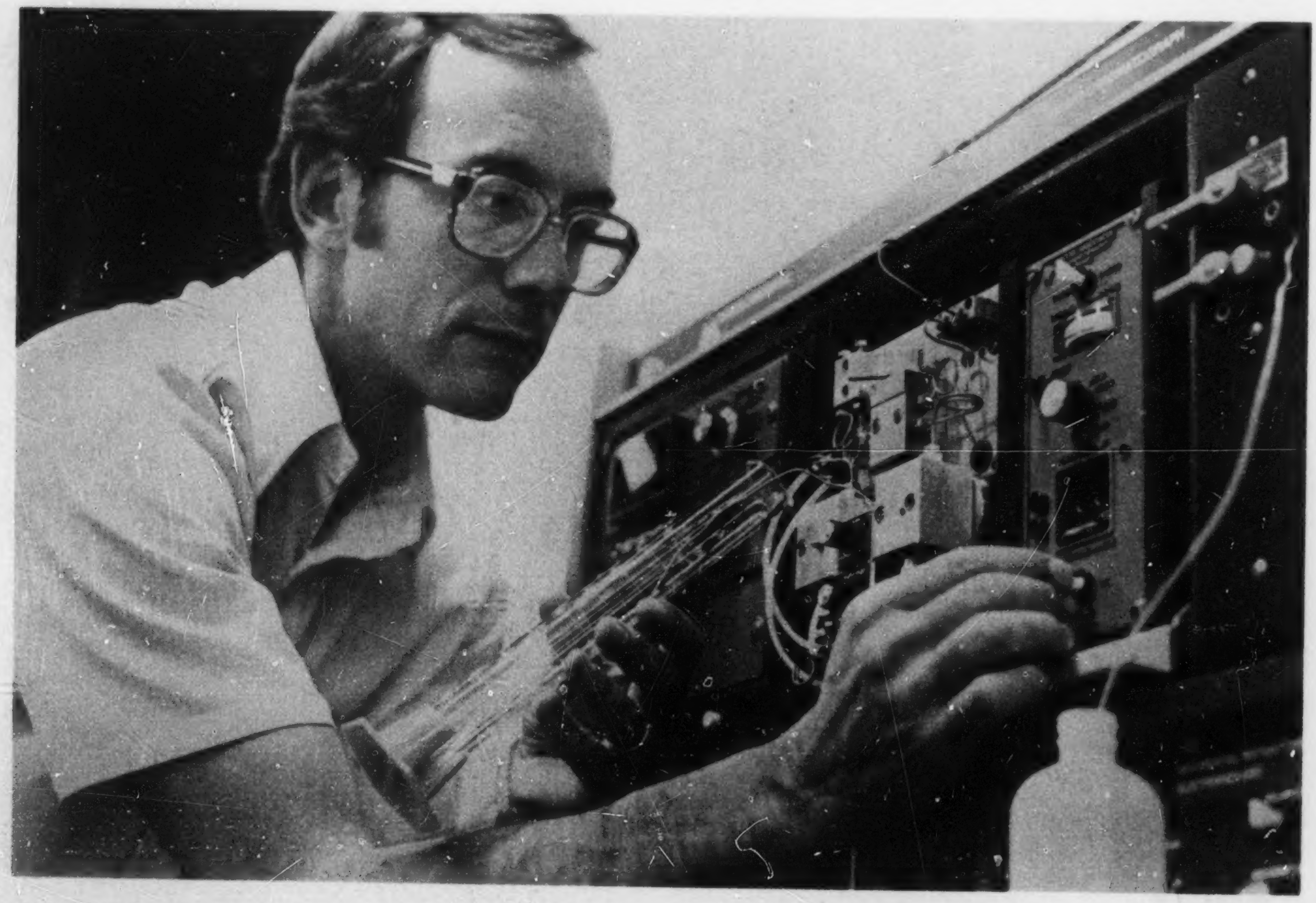
Dr. Kuehn is currently collaborating with Dr. Sekar to determine whether ODC and the newly discovered enzyme are indeed target proteins for interferon's action. "Preliminary experiments support our conjecture," Dr. Kuehn says.

Rapid inhibition of ODC by polyamines has been known for some time, but the intermediate step involving ODC kinase was not known. Dr. Kuehn's discovery of this enzyme explains the mechanism of the ODC inhibition reaction. The new protein kinase catalyzes the transfer of phosphate from adenosine triphosphate (ATP) to ODC in a reaction that is polyamine-dependent.

Polyamines are found in highest concentration in rapidly proliferating tissues where they have been related to the control of RNA metabolism. ODC's function in the body is to catalyze the first step in polyamine synthesis in which the amino acid ornithine is decarboxylated to the amine putrescine.

Dr. Kuehn and his colleagues discovered that ODC's activity in *Physarum* is regulated by reversible phosphorylation. Phosphorylation by ODC kinase inactivates ODC. Dephosphorylation by phospho-ODC phosphatase reactivates the enzyme.

Polyamines are essential in the phosphorylation reaction. Spermidine and spermine acting syn-



ergistically are especially active in this respect. These amines are formed from the putrescine produced in the first step of the biosynthetic sequence. The researchers found that this unique polyamine-dependent reaction takes place on the ribosomal DNA minichromosome of the nucleolus in *P. polycephalum.*

Inactivated ODC also seems to play at least one important role in the cell. Earlier work by Dr. Kuehn's group showed that phosphorylated ODC had numerous properties indicating a specific regulatory role in ribosomal RNA (rRNA) gene transcription in the nucleolus. It was found to be a component of a deoxyribonucleoprotein complex isolated from the nucleoli of *P. polycephalum*, where it specifically stimulates ribosomal RNA gene transcription by RNA polymerase I within the deoxyribonucleoprotein complex. This discovery, made in collaboration with researchers at the University of Berne in Switzerland, may be especially significant in connection with interferon's antiviral action, which involves the induction of a new viral inhibitory protein in the cell.

"It seems most likely that phosphorylation of ODC serves a much broader role than merely preventing and controlling the biosynthesis of the polyamines," says Dr. Kuehn.

For further information, see:
1. Daniels, G. R., Atmar, V. J., and Kuehn, G. D., Polyamine-activated protein kinase reaction from nuclei and nucleoli of *Physarum polycephalum* which phosphorylates a unique M_r 70,000 nonhistone protein. *Biochemistry* 20:2525-2532, 1981.
2. Atmar, V. J., and Kuehn, G. D., Phosphorylation of ornithine decarboxylase by polyamine-dependent protein kinase. *Proceedings of the National Academy of Sciences, USA* 78:5518-5522, 1981.

Research into ornithine decarboxylase regulation and interferon action is supported in part by the Minority Biomedical Support Program of the NIH Division of Research Resources and by the National Institute of General Medical Sciences.

An hereditary disorder very similar to human motor neuron disease is being studied in a colony of Brittany spaniels at Johns Hopkins University School of Medicine.

Like motor neuron diseases such as amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease, which affects humans, the canine disease damages motor neurons that innervate muscles. When these neurons malfunction or die, weakness, muscle atrophy, and eventually paralysis result. Intelligence and sensory functions are not significantly altered.

The disorder, called hereditary canine spinal muscular atrophy (HCSMA), was discovered in 1976 by Dr. Linda Collins Cork, a veterinary pathologist at Johns Hopkins. Dr. Cork first recognized the disease in a 9-month-old Brittany spaniel pup which was weak and had muscle atrophy. Breathing was labored, and the pup could walk only a few yards before becoming exhausted. Autopsy confirmed the diagnosis of motor neuron disease. Dr. Cork established a colony of Brittany spaniels with the disease by breeding

Dr. Glenn D. Kuehn, professor of chemistry at New Mexico State University: ***quantitating polyamines with a high-performance liquid chromatograph.***

Dr. Valerie Atmar, postdoctoral research assistant, concentrating a purified ODC kinase solution: ***only small amounts of ODC kinase can be extracted and purified from biological specimens.***

Ms. Janet Klein, graduate research assistant: ***analyzing polyamine analogues used to study the interaction of ODC kinase and ODC.***

kindred of the original dog.

Five generations of Brittany spaniels later, Dr. Cork and her colleagues have shown that the disease is inherited as a dominant trait and that it varies in severity of expression. By selective breeding, certain dogs now develop the disease late in life and with symptoms similar to human motor neuron disease, such as ALS. In humans ALS usually strikes between the ages of 40 and 70. Once stricken, most patients live only a few years.

Another form of motor neuron disability, Werdnig-Hoffmann disease, affects infants. Estimated incidence is 1 in 20,000 live births. The disease is inherited, and the gene frequency has been estimated at 1 in 60 to 80 in the general population—second only to cystic fibrosis in frequency. Like ALS, the disease is fatal.

Selective breeding in the HCSMA colony has produced pups that develop a rapidly progressive form of HCSMA which causes complete paralysis by 3 to 4 months of age, a condition similar to Werdnig-Hoffmann disease.

Another less severe form of spinal muscular atrophy called Kugelberg-Welander syndrome occurs in childhood. Children with this disease develop progressive weakness and paralysis over a period of several years. Some dogs in the HCSMA colony have a similar disease pattern. Thus, the canine model resembles the human motor neuron disease in its genetics, clinical features, and pathologic changes. Because of the striking clinical and pathologic similarities, it offers an opportunity to conduct investigations not possible in humans.

Little is known about the causes, origin, and development of motor neuron disease in humans or why it selectively attacks the motor neurons. A major stumbling block to scientists has been that many of the genetic, pathologic, and biochemical studies required are not possible in people. Together with Drs. John W. Griffin, Robert J. Adams, and Donald L. Price of Johns Hopkins, Dr. Cork has initiated such investigations to help understand the progression of the diseases.

Research on HCSMA has been supported by the Amyotrophic Lateral Sclerosis Society of America, The H. Burnett Robinson Memorial Fund, the National Institute of Neurological and Communicative Disorders and Stroke, and the Animal Resources Program and the Biomedical Research Support Grant Program of the NIH Division of Research Resources.

Models for human diseases: ***some Brittany spaniels at Johns Hopkins University have a disorder similar to human motor neuron disease; others serve as models for complement (C3) deficiency.***

A genetically determined deficiency of the third component of complement (C3) has been identified in a colony of Brittany spaniels. Although a few humans have shown a genetically determined C3 deficiency, the researchers say this is the first time it has been found in an experimental animal.

The investigators believe these dogs will prove valuable in generating a better understanding of the significance of C3 deficiency as well as elucidating the role of C3 in a variety of immunologic reactions in normal individuals. The researchers are currently working to understand the genetic basis for the deficiency.

Dr. Jerry A. Winkelstein, an immunologist at the Johns Hopkins University School of Medicine, and Dr. Linda Collins Cork identified the deficiency, together with a team of Hopkins investigators composed of Drs. Diane E. Griffin, John W. Griffin, Robert J. Adams, and Donald L. Price.

The deficiency was uncovered while genetic studies, including selective inbreeding, were being carried out in a colony of dogs for the study of an inherited neurologic disease resembling motor neuron diseases in humans. During the course of these studies, a number of the puppies developed severe bacterial infections. This prompted an investigation of their immune systems, which, in turn, led to the discovery of the C3 deficiency.

The deficiency is inherited as an autosomal recessive trait, a mode of inheritance that is not sex-linked. Heterozygotes, which carry the trait, show intermediate levels of C3. In further breeding studies, the C3 deficiency trait will be separated from the trait for the motor neuron disorder.

The complement system is a series of proteins that, when activated, participates in a variety of protective and inflammatory reactions. One of the complement proteins, C3, seems to play an essential role in these immunologic functions. One of the main functions of C3 is to attach to invading microorganisms, such as bacteria, and thereby render them more susceptible to ingestion by white blood cells.

"Clinical findings have shown that C3-deficient patients are more susceptible to bacterial infections than those individuals with normal immune systems," Dr. Winkelstein notes. In addition to infections such as pneumonia and meningitis, these patients may also have fever, skin rash, and joint pains. Like the human patients, the C3-deficient spaniels are unusually susceptible to infections, but as yet they have not shown other symptoms found in C3-deficient humans.

Research into C3 deficiency in Brittany spaniels has been supported by the Amyotrophic Lateral Sclerosis Society of America, the National Science Foundation, the National Institute of Allergy and Infectious Diseases, and the Animal Resources Program and the Biomedical Research Support Grant Program of the NIH Division of Research Resources.

Research Service

Human Hemoglobin Available for Research

Human hemoglobin, stroma free, is currently being made available for evaluation as a blood substitute to qualified investigators by the Division of Blood Research, Letterman Army Institute of Research, Presidio of San Francisco, California.

The product is intended mainly for studies designed to develop a field-adaptable blood substitute preparation for resuscitation. Such a preparation should be suitable for treating hypovolemic shock and anemic hypoxia. Stroma-free hemoglobin will be available for research into the efficacy, safety, and modification of hemoglobin. The product will not be supplied for the treatment of human beings.

Since the supply is limited, requests will be given priority on the basis of conformance with the intended use of the product. A protocol and curriculum vitae of the principal investigator, the purpose of the study, a statement that the material will not be used in human beings, the amount required, when it is needed, and other pertinent information must be stated in the request. The special hemoglobin is free of charge except for shipment expenses.

Interested and qualified investigators should contact:

LTC Robert B. Bolin, M.D., USA, MC
Division of Blood Research
Letterman Army Institute of Research
Presidio of San Francisco,
California 94129
Telephone (415) 561-5875

New Directory to Resources Of Biomedical and Zoological Specimens

The Registry of Comparative Pathology has published a new directory, "Resources of Biomedical and Zoological Specimens." The directory lists 114 collections located in the United States and foreign countries. A free copy of the directory may be obtained from the Registry of Comparative Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306.

Meetings

Symposium on Neoplastic Disorders

The Eighth Annual Symposium on Diagnosis and Treatment of Neoplastic Disorders—Medical, Surgical, and Radiotherapeutic Aspects will be held March 18 to 20, 1982, at the Johns Hopkins Medical Institutions in Baltimore, Maryland. It will focus on new approaches to cancer treatment, therapy of specific malignancies, and management of the complications of cancer. The program will include minisymposia on breast cancer, chronic myelocytic leukemia, Hodgkin's disease, and female germ cell tumors. Modifiers of biologic response, hyperthermia, radioimmunoglobulin therapy, new anticancer drugs, and in vitro drug testing will also be highlighted. New approaches to viral infections, management of urologic complications, and psychologic management of cancer patients and their families will be discussed, relevant to their role in total patient care. The program will conclude with an overview on the management of ovarian cancer at the conjoint clinic.

For further information contact the Program Coordinator, Continuing Education, Turner Auditorium, Room 22, 720 Rutland Avenue, Baltimore, Maryland 21205, telephone (301) 955-5880.

February 26-27, 1982

Title: *Symposium on Pediatric Oncology*
Location: *London, England*
Contact: *Meetings Secretary*
Royal College of Radiologists
38 Portland Place
London, WIN 3DG England

March 3-5, 1982

Title: *3rd Annual Meeting of the Society of Behavioral Medicine*
Location: *Chicago, Illinois*
Contact: *The Society of Behavioral Medicine*
P.O. Box 8530
University Station
Knoxville, Tennessee 37996
Telephone (615) 964-5164

March 22, 1982

Title: *Symposium on the Effects of Ultrasound on the Fetus*
Location: *New York, New York*
Contact: *L. Stanley James, M.D.*
Professor of Pediatrics
Columbia University, College of Physicians and Surgeons
630 West 168th Street
New York, New York 10032
Telephone (212) 694-5827

April 14-16, 1982

Title: *28th Annual Meeting of the American Society for Artificial Internal Organs*
Location: *Chicago, Illinois*
Contact: *K. K. Burke*
Executive Director
ASAIO National Office
2200 North Federal Highway
Suite 203
Boca Raton, Florida 33432
Telephone (305) 391-8589

May 3-8, 1982

Title: *2nd Congress on Hemotherapy and Immunohematology*
Location: *Buenos Aires, Argentina*
Contact: *Secretariat*
Sarmiento 1562, 4E
1042 Buenos Aires, Argentina

May 4-7, 1982

Title: *International Symposium on the Secretory Immune System*
Location: *New York, New York*
Contact: *Conference Department*
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021
Telephone (212) 838-0230

May 6-7, 1982

Title: *The American Red Cross 14th Annual Scientific Symposium: Massive Transfusion in Surgery and Trauma*
Location: *Washington, D.C.*
Contact: *Symposium Coordinator*
American Red Cross Blood Services Laboratories
9312 Old Georgetown Road
Bethesda, Maryland 20814
Telephone (301) 530-6040

May 6-9, 1982

Title: *64th Spring Meeting of the Association of Clinical Scientists*
Location: *Santa Monica, California*
Contact: *F. W. Sunderman, Jr. M.D.*
Department of Laboratory Medicine
University of Connecticut School of Medicine
263 Farmington Avenue
Farmington, Connecticut 06032
Telephone (203) 674-2328

May 7-8, 1982

Title: *Annual Meeting of the American Society for Clinical Nutrition*
Location: *Washington, D.C.*
Contact: *G. M. Knight*
Executive Officer
American Society for Clinical Nutrition
9650 Rockville Pike
Bethesda, Maryland 20014
Telephone (301) 530-7110

May 15-18, 1982

Title: *77th Annual Meeting of the American Thoracic Society*
Location: *Los Angeles, California*
Contact: *Dr. Robert M. Senior*
ATS Annual Meeting Committee
American Thoracic Society
1740 Broadway
New York, New York 10019
Telephone (212) 245-8000 x570

May 18-21, 1982

Title: *5th International Conference on Prostaglandins*
Location: *Florence, Italy*
Contact: *Fondazione Giovanni Lorenzini*
Via Monte Napoleone 23
20121 Milan, Italy

May 19-21, 1982

Title: *Conference on the Endothelium*
Location: *New York, New York*
Contact: *Conference Department*
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021
Telephone (212) 838-0230

May 19-22, 1982

Title: *International Symposium on the Conservation of the Lion-tailed Macaque*
Location: *Baltimore, Maryland*
Contact: *Robert H. Johnson*
Baltimore Zoological Society
Druid Hill Park
Baltimore, Maryland 21217
Telephone (301) 484-2630/2413

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Research Resources Reporter

March 1982, Volume VI, No. 3

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By
Donald W. McKinstry, Ph.D.

Treating Immune Defects: Research Seeks Ways To Build Natural Defenses

For the first 11 months of his life, Jerry was confined to a germ-free room. He was freed when doctors performed a bone marrow transplantation that established in the child a defense system against infection, a system that had been all but nonexistent due to a congenital defect called severe combined immunodeficiency disease (SCID).

Babies with SCID are deficient in both parts of the immune system—antibody and cellular immunity—which leaves them defenseless against infection. Without proper care, the disease is fatal early in life.

It was the germ-free room, constructed in the General Clinical Research Center (GCRC) at Wyler Children's Hospital of the University of Chicago, that kept

© Chicago Sun-Times, photo by Kathleen Reeve, reprinted with permission

Jerry Watkins happy to be going home after spending his first 11 months in a germ-free room at Wyler Children's Hospital at the University of Chicago: ***Jerry was isolated from disease-causing germs until his defense system could be rebuilt following a bone marrow transplantation for severe combined immunodeficiency disease (SCID) which leaves babies defenseless against microbial infection.***

HE 20. 3013/6:

IMMUNE DEFECTS *continued*

Jerry alive before the marrow transplant and afterward, during the time when he needed to build natural defenses against germs.

"Transplantation is technically a simple procedure and could be done at most large medical centers," says Dr. Richard M. Rothberg at the Wyler Children's Hospital Clinical Research Center. "But because we don't have a definitive therapy for patients who do not have compatible bone marrow donors, research into the mechanism of the disease is vital and can be justified with a germ-free environment for the patient. With a sterile environment it is possible to delay transplantation after diagnosis, because the child's well-being is protected."

The germ-free room is part of a comprehensive treatment program at the Wyler GCRC, which is specifically designed for children with SCID. A similar room is maintained to support patients receiving bone marrow transplants by Dr. Robertson Parkman and his colleagues at the GCRC in Children's Hospital Medical Center in Boston. They, like the surgeons at Wyler Hospital, depend on the room to house children with SCID.

Everything in these rooms must be sterile, from the furnishings and medical equipment to the child's toys. Even the air the patient breathes is sterilized by special filters. Visitors are limited to parents and even they must wear sterile outer coverings—gowns, face masks, slippers, gloves, and hair covering. Even after a successful transplantation, the patient must be confined to this sterile environment for several months to give the body time to develop adequate resistance to infection.

The support staff of nurses, aides, dietitians, and maintenance personnel at GCRC's is essential in caring for SCID patients. Each center has its own kitchen and its staff of dietitians observe elaborate sterile precautions in preparing and serving low-bacteria food to the bone marrow transplant patients.

The programs at Wyler and the Boston Children's Hospital are two of several programs focused on immune defects at GCRC's across the country. The Boston Children's Hospital team, consisting of Drs. Parkman, Fred Rosen, Raphael Levey, David Nathan, and Joel Rappeport, has had considerable experience and success with bone marrow transplantations dating back to 1966. Successful transplantations have been performed for patients with both SCID and aplastic anemia, a less severe though still debilitating disease of the bone marrow.

The doctors have also succeeded in curing other patients with immune defects less serious than SCID. Recently four patients with Wiskott-Aldrich syndrome, which results from defects in both the immune system and platelets and is characterized by eczema, were successfully treated by the team with bone marrow transplants from immunologically compatible siblings. Previous attempts to correct these deficiencies by bone marrow transplantation were unsuccessful.

The reseachers believe that the successful outcome with Wiskott-Aldrich patients was due, in large measure, to pretransplantation treatment with antihuman thymocyte serum and limited total body irradiation, which is designed to prevent overgrowth of the donor stem cells by the recipient's hematopoietic stem cell population. All patients had prompt and complete engraftment of donor cells with no evidence of chronic graft-versus-host disease. The patients ranged in age from 4 months to 4 years. All four children continue to demonstrate normal immune and hematopoietic functions.

During the course of treatment, the patients were protected from microbial infection by isolation in laminar flow units in the Boston GCRC. These units drastically reduce exposure to germs. Other precautions include a diet low in bacterial content, use of non-

Photo courtesy of The Children's Hospital Medical Center, Boston, Massachusetts

Research Resources
Reporter

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Center

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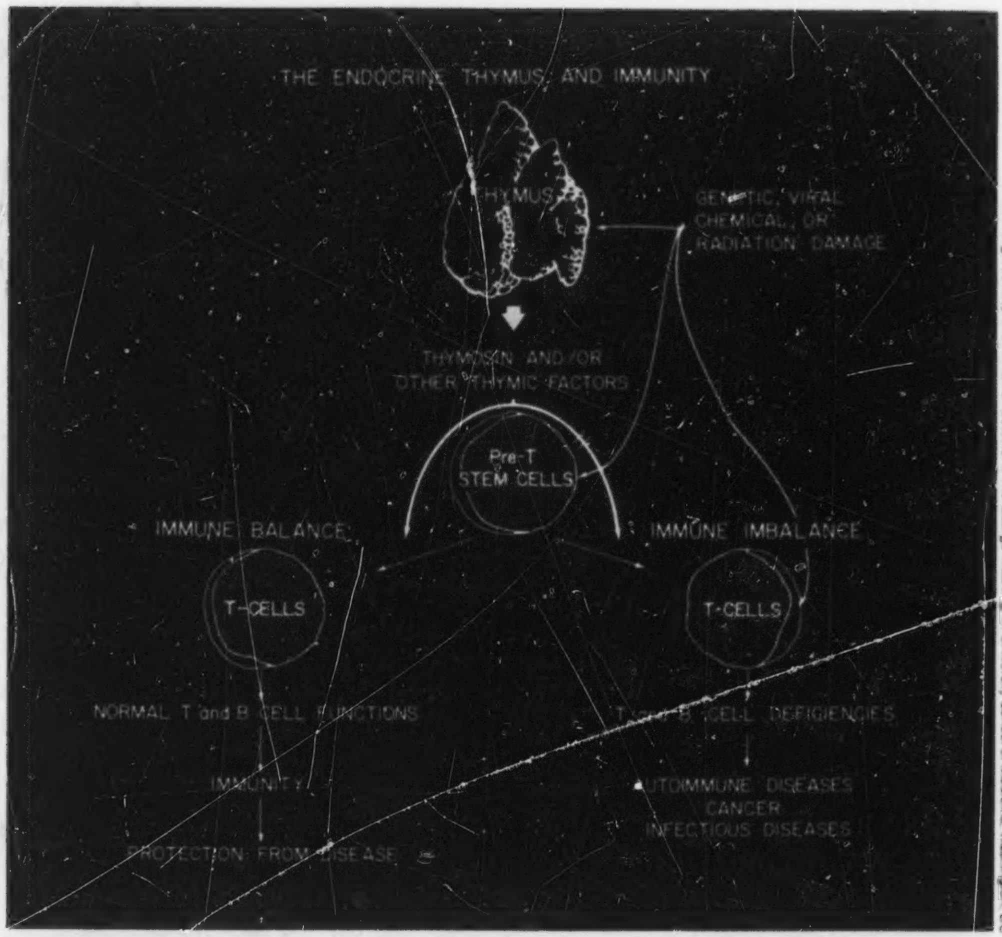
Dr. Robertson Parkman, Children's Hospital Medical Center, Boston, Massachusetts: ***typing tissue for a prospective bone marrow transplantation.***



Role of the thymus in the immune system.

Drawing courtesy of Dr. Allan L. Goldstein, The George Washington University School of Medicine, Washington, DC

absorbable antibiotics to inhibit bacteria in the intestinal tract, and antibacterial skin care.

This rigorous isolation is required, particularly for children born with SCID, the most severe type of immune defect, because even the slightest infection can lead to death. SCID children are so vulnerable because their bone marrow cannot manufacture effective white blood cells, which normally fight off infection. The thymus, spleen, and lymph nodes, which also participate in the immune system, are also poorly developed and of little use.

The main problem with bone marrow transplantation, the traditional method of treatment for SCID, is finding a donor whose bone marrow is compatible with that of the patient. If the tissues of a marrow transplant donor are not exactly the same immunologically as those of the recipient, the graft usually will not take. Or, even worse, the transplanted cells that repopulate the marrow may produce antibodies and T lymphocytes that attack the patient's own tissues—graft-versus-host disease (GVHD)—which sometimes causes death. Methotrexate, an anticancer drug, is used to combat GVHD. However, in spite of the best drugs and methods for donor-recipient matching, GVH reactions occur in about 50 percent of bone marrow transplantation patients.

Jerry was lucky to have a sister whose marrow was compatible with his own. After careful tissue typing, Dr. Rothberg and his colleagues Drs. Herbert Krantman and Eugene Posnock of the University of Chicago Medical School believed that the transplantation had a good chance of succeeding. But Dr. Rothberg warned the parents that the transplant could react severely against Jerry's own body and even cause death.

On the day of the transplantation, the surgical team of Drs. Frank P. Stuart and Beverly Ketel aspirated marrow from the donor's pelvic bones while the donor was under anesthesia. Then they injected the marrow into Jerry's abdomen. Transplanted marrow cells are believed to be transported by the circulatory system to bone cavities and, if accepted, they repopulate the defective marrow with cells that produce normal, effective white blood cells.

The transplantation was successful. After a short period of fever, rash, and inflammation, signs of a functioning immune system were detected. Doctors can usually tell if the marrow has been accepted within 14 days. Jerry had to be shielded from infection for several months longer.

Jerry's exposure to the outside world and its germs was gradual. After each encounter his immune response was reevaluated. Finally, 7 months after the transplantation, he was ready to go home. Dr. Rothberg believed there was a good chance that Jerry would be able to lead a normal life. Now, 2 years later, results of periodic examinations confirm Dr. Rothberg's optimism.

IMMUNE DEFECTS *continued*

Photo by Eolan Bates, courtesy of University of California, San Francisco News Services

Jerry is doing well. An earlier transplant patient, a young girl, is also doing well 3 years after receiving a bone marrow transplant for SCID.

The two children are unusually lucky. Compatible bone marrow donors are hard to find. Even with a well-matched sibling donor, some transplantations are unsuccessful. Dr. Parkman believes that this is due to the complexity of tissue itself. According to Dr. Parkman, some antigens may be missed despite rigorous tests.

Candidates for bone marrow transplants and their prospective donors are tested for compatibility by three procedures—HLA typing, mixed leukocyte culture (MLC) test, and cytotoxicity testing. The genetically determined HLA antigens on the cell surface are of major importance in transplantation. There is one chance in four that transplanted cells from a sibling of the patient will not be recognized as a foreign agent and that the graft will take.

The MLC test indicates in the test tube what is likely to happen in the body when the donor's marrow cells are transplanted. If the donor cells are different immunologically from those of the patient, they will be stimulated to grow, indicating a poor match. If there is no growth in the mixture, the transplantation may be successful. The cytotoxicity test is another in vitro procedure designed to determine if donor or recipient cells will kill one another.

An approach that bypasses the problem of tissue matching is being used by Dr. Rebecca H. Buckley and her colleagues at Duke University Medical Center in Durham, North Carolina. They have achieved an active immune system in some SCID children by transplanting fetal liver cells.

The first patient to receive the treatment was 13 months old when he was given an intra-abdominal injection of liver cells from an 8-week-old fetus. The transplantation was successful, and the boy is now alive and doing well 8 years after the injection. The thymus-dependent (T cell) component of his immune system is functioning almost normally, although he receives gamma globulin injections to supplement his own production of antibodies. His only problem now is chorioretinitis, which resulted from a cytomegalovirus infection.

Liver cells obtained from a fetus prior to 12 weeks' gestation contain hematopoietic stem cells that produce white blood cells which do not have the capacity to cause fatal GVHD. Consequently, a tissue-compatible donor is not necessary—a great advantage when fighting a disease in which early treatment is essential and when a compatible donor may not be available. The other advantage of the fetal liver cell treatment is its apparent safety.

It has not had a high success rate with the limited number of patients treated to date, some of whom were very sick when brought in for treatment. Three SCID patients have been treated at the Duke GCRC since the first case. One, a half-brother of the first patient, was given six liver cell transplants over a 40-month period, but the treatment was only minimally effective, Dr. Buckley says. The patient died of a lymphoma. However, tissue typing studies revealed the tumor to be of patient origin, not from donor cells. The other two patients did not respond clinically to the treatment. These patients were very sick when they came for treatment and died from infections. Dr. Rothberg also has treated a 6-month-old SCID patient at the University of Chicago GCRC with liver cells from a 6-week-old fetus. Although the boy's immune system functioned after the treatment, he died at age 3. The cause of death, according to Dr. Rothberg, was either chronic GVHD or an inability to absorb nutrients via the intestines, which may be a basic defect associated with SCID.

Evaluation of the procedure requires more extensive trials. Two patients are now awaiting fetal liver cell implants at the Duke GCRC.

The need for effective therapy has led researchers to try diverse methods. One is the use of thymic epithelium implants, which have been used successfully to treat SCID and several other immunodeficiency disorders. The thymus plays an important role in immunity, par-

Debra and Don Whitmore with children Justin and Nicole: ***both children recovered from a serious immunodeficiency disorder when treated with large doses of biotin.***

Photo by Susan Paddor Lukes © 1981

Drs. Morton J. Cowan (left), Diane W. Wara, and Arthur J. Ammann, department of pediatrics, University of California, San Francisco: ***using thymosin to treat patients with immunodeficiency disorders due to malfunction of the thymus gland.***

ticularly in influencing the maturation, proliferation, and immunologic competence of T lymphocytes.

Thymus tissue for transplanting is obtained through biopsy from children undergoing open-heart surgery. The tissue fragments are cultured for about 2 weeks to obtain a suspension consisting primarily of epithelial tissue. The epithelial fragments are then implanted into the patient's leg muscle, where they may become established, induce the formation of new blood vessels, and grow to about the size of an air-gun pellet.

"Each thymus fragment then functions as a miniature thymus," says Dr. Richard Hong of the University of Wisconsin Medical School, who has performed 48 thymic epithelium implants at various centers, including GCRC's at the University of California in Los Angeles, the University of California in San Francisco, and Johns Hopkins University, Baltimore.

Approximately 25 percent of the patients receiving these implants have responded favorably and are now in good health. The first patient received an implant 4½ years ago. Dr. Hong believes that the 25 percent response rate does not truly indicate the potential value of the treatment, since many patients were not brought for treatment until they were very ill.

Since no foreign white blood cells or lymphocyte-producing tissue is implanted in the thymic epithelium treatment, a compatible donor is not necessary, and graft-versus-host disease does not develop. A few patients receiving thymic implants later developed cancer, but Dr. Hong says that followup studies indicate that these malignancies were not directly related to the implants. Patients with immunodeficiency, he says, are particularly vulnerable to cancer.

An extract of the thymus gland, called thymosin, has been used successfully over the past several years in the treatment of certain immunodeficiency disorders. Thymosin was first tried clinically in 1974 by Drs. Arthur J. Ammann and Diane W. Wara at the University of California in San Francisco. The patient was a 5-year-old girl. She was born with a faulty thymus and a deficient immune system.

The young girl responded to the therapy with marked clinical improvement and a pronounced increase in her T cell population. Today, 7 years after the initial treatment, she is doing well and is no longer dependent on thymosin, Dr. Wara says, even though her T cell level is somewhat low. She attends school and has weathered common childhood infections including chickenpox, a disease that often kills children with severe immunodeficiency syndromes.

Since 1974 Drs. Ammann and Wara have treated 34 patients afflicted with a wide variety of immunodeficiency disease. About half the patients have responded favorably to thymosin treatment. Variation in the response appears

IMMUNE DEFECTS *continued*

to have been due to the nature and extent of the immune defect. The majority of patients with DiGeorge's syndrome, for example, responded favorably to the treatment, whereas classical SCID patients with severe bone marrow deficiency failed to respond. DiGeorge's patients have a very small or immature thymus and an impairment of cell-mediated immunity, but they do not lack stem cells in the bone marrow.

Thymosin is a mixture of thymus hormones, some of which increase the number, maturation, and function of T lymphocytes. The specific functions of the various individual factors are not known at present. Dr. Wara believes that for some conditions, treatment with different thymus factors in a specific sequence may be necessary.

The thymosin fraction 5, used by Drs. Ammann and Wara to treat their first patient, was provided by Dr. Allan L. Goldstein of the George Washington University School of Medicine and Health Sciences in Washington, D.C. Dr. Goldstein, in collaboration with the late Dr. Abraham White, discovered thymosin at the Albert Einstein College of Medicine in 1965.

Dr. Goldstein and Dr. Teresa L. K. Low have been working for several years in extracting, purifying, and characterizing the various hormones in the thymosin fraction 5 family. Thymosin alpha$_1$, the first peptide they isolated and purified and for which they determined the sequence of amino acids, is 10 to 1,000 times as active as the parent fraction in inducing T cell responses and in amplifying the immune response. It has now been produced synthetically and is available commercially, although the supply is limited. Most recently the gene for thymosin alpha$_1$ was synthesized and alpha$_1$ was produced via recombinant DNA technology. It is being tried clinically in the treatment of several forms of cancer as well as for treating immunodeficiency syndromes associated with thymic malfunction.

A second thymosin peptide, termed thymosin beta$_4$, has also been purified and its sequence determined by Dr. Goldstein and his colleagues. This peptide, in addition to having potent effects on immature T cells, has a direct effect on the hypothalamus and the pituitary and can stimulate the production of luteinizing releasing factor and luteinizing hormone. Thymosin fraction 5 is currently being evaluated in the United States in patients with primary immunodeficiency diseases, in cancer patients, and in patients with autoimmune disorders, particularly systemic lupus erythematosus.

Dr. Goldstein says that thymic hormones also have a potentially important role when there is infectious disease and in aging when there is a decline in T cell immunity. In China, patients with hepatitis, leprosy, and other infectious diseases have been reported to show significant improvement clinically after treatment with thymosin fraction 5. Dr. Goldstein hopes that alpha$_1$, beta$_4$, or other thymus hormones now being isolated and purified will be effective in those disorders that do not respond to treatment with the partially purified thymosin fraction 5.

Finding a cure for SCID is particularly difficult because this type of disorder, like cancer, may be a group of disorders rather than a single disease. Some patients have few or no lymphocytes, whereas others have B but not T lymphocytes. Most, however, are severely deficient in T cells. Some patients have lymphocytes that lack an essential enzyme or require an added nutritional factor. Some SCID patients lack the enzyme adenosine deaminase (ADA), a discovery made at the Albany Medical Center GCRC.

Several years ago, Dr. Stephen H. Polmar of Case Western Reserve University School of Medicine and his colleagues successfully treated a 7-month-old SCID baby with transfusions of irradiated normal red blood cells (RBC's) containing the enzyme. In addition to restoring T and B lymphocyte levels to normal, a "thymus shadow" appeared in the chest X-ray after 2 weeks, and thymic hormone levels were also in the normal range by the 4th week of therapy.

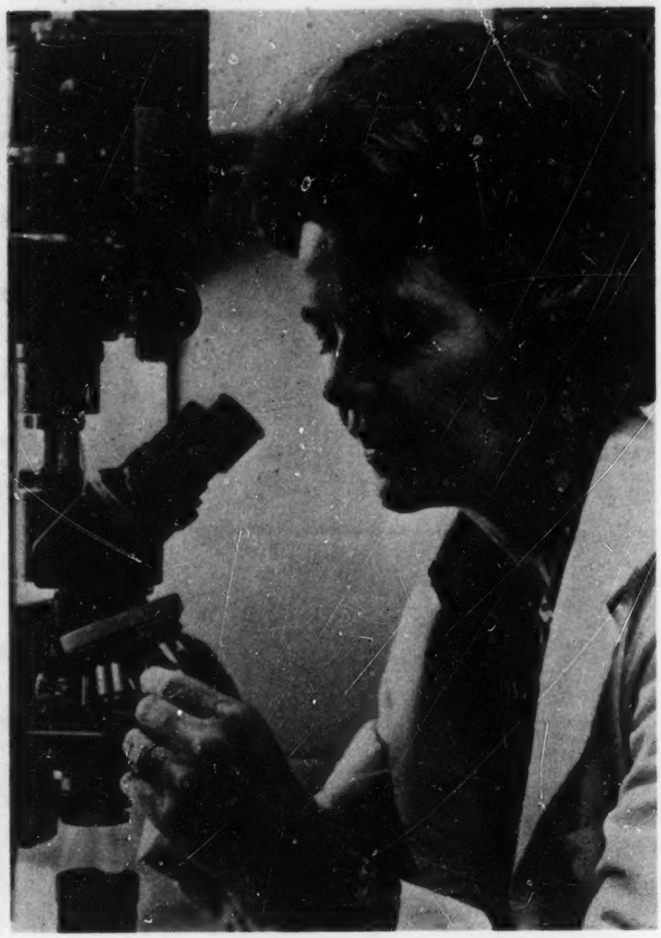


Photo courtesy Public Relations Department, Duke University Medical Center, Durham, North Carolina

"The patient was treated regularly for almost 6 years, was free from significant infection, and maintained good physical growth and intellectual development," says Dr. Polmar. This apparently was the first time that RBC enzyme therapy had been attempted in a human metabolic disease, he adds. The patient died, however, of a chickenpox infection after 6 years of therapy.

Dr. Polmar and coworkers had been encouraged to attempt RBC enzyme replacement therapy on the basis of in vitro experiments. The researchers found that the patient's lymphocytes, when cultured in the presence of calf intestine ADA or human RBC's, regained their ability to proliferate when stimulated with mitogens, substances that induce cell division.

Ten additional ADA-SCID patients have been treated with RBC transfusions during the past several years. Five patients responded favorably, showing freedom from infections and good physical growth and development. The other five, however, showed no immunologic response. Immunologic studies on both groups of patients revealed that those who benefited from the treatment had some marginal B and T cell function at the time of diagnosis, where as those who failed to respond had no measurable immunologic function to start with.

"Enzyme replacement therapy

Dr. Rebecca H. Buckley examining specimen at Duke University Medical Center in Durham, North Carolina: ***bypassing the problem of tissue matching to restore immune response with transplanted fetal liver cells.***

results in partial restoration of immune function," Dr. Polmar says. "Those treated can live normal lives for 6 or more years but are not completely normal from an immunologic standpoint. Those not treated will die by 2 years of age."

Dr. Polmar points out that RBC-ADA replacement therapy carries some risk for the patient. Transfer of large numbers of RBC's over a period of time may result in chronic iron overload. There is also the possibility of sensitization to minor blood group antigens and a slight risk of hepatitis. "The GCRC is of special value during the transfusion since it is an exchange transfusion in which blood must be withdrawn from the patient prior to the administration of normal red blood cells," says Dr. Polmar. "There must be intensive monitoring during the transfusion as well as careful long-term observation following the transfusion, for any evidence of iron overload or other adverse reactions."

Use of purified ADA would eliminate these risks, but Dr. Polmar says the supply available up to now has not been sufficient for therapy. Also, the purified enzyme, when injected intravenously, is broken down within minutes in the bloodstream. A new procedure developed recently involves loading the patient's own RBC's with ADA to treat the disorder, thereby eliminating the risk from donor blood.

Dr. Polmar points out that ADA-SCID can be diagnosed before birth with cells collected by amniocentesis. Thus, the physician and family can be prepared for promptly treating the condition after the birth of the baby.

Even if a bone marrow transplantation is possible, RBC enzyme replacement therapy can be used to reduce the risk to the patient until the transplantation can be performed, according to Dr. Polmar. The RBC treatment also improves the health of the patient, which is generally poor, he says, because ADA deficiency affects the function of many different types of cells in the body in addition to cells of the immune system.

A child with SCID may also have another metabolic defect, hereditary biotin dependence, which adversely affects the immune system. This defect was discovered several years ago by researchers at the University of California in San Francisco. Drs. Seymour Packman, Morton J. Cowan, and their colleagues identified the condition in one of three siblings with serious immunodeficiency disorders. Without large amounts of biotin, metabolic disturbances occurred and damaged the immune system. Two children died from infections before therapy could be started. The third child, a girl 18 months of age, recovered rapidly when treated with large doses of biotin. She is doing well today, 3 years after the initial treatment, but must continue taking biotin regularly. Prior to admission to the hospital for treatment the child suffered repeated *Candida* and other microbial infections.

The nature of the metabolic defect was suggested by lactic acidosis and abnormally high levels of organic acids in the urine. These findings indicated a deficiency in biotin-dependent carboxylase enzymes. A variety of standard in vitro and in vivo tests uncovered the immunodeficiency.

In addition to ADA-SCID and the biotin-associated disorder, three other inherited metabolic defects have been linked to immunodeficiencies. In these disorders, the enzymes purine nucleoside phosphorylase, 5'-nucleotidase, NADPH oxidase, and G6PD are involved.

Recent success with RBC enzyme replacement therapy, though limited, has encouraged researchers that similar regimens can be found for SCID children and others with immunodeficiencies. Still the search for other modes of therapy continues. Most physicians studying the problem agree that better results may be had by combining several approaches. Bone marrow transplantation and RBC treatment may restore immunologic function in ADA-SCID, but it is hard to find compatible donors. Fetal liver transplantation has been used with some success, and thymus implants have also been attempted, but more clinical studies are necessary to evaluate these procedures. **R**

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Research into SCID and other immunodeficiency disorders, which was described in this article, was supported in part by the General Clinical Research Centers Program of the NIH Division of Research Resources, the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, the National Institute of Child Health and Human Development, the National Institute on Aging, the March of Dimes Birth Defects Foundation, Hoffmann-LaRoche, Inc., and the University of Wisconsin Cultured Thymus Fund supported by M. Reierson, W. Willoughby, R. Raetz, and friends.

Research Focus

Hypertension And Malfunctions Of the Immune System

Some aspects of hypertension may result from malfunctions of the immune system, according to investigators at Rutgers University in Newark, New Jersey.

"Genetics plays a strong role in both human and animal hypertension," says Dr. Helen R. Strausser, a physiologist at Rutgers. "These genetic factors may be linked to the immune response, since in both human and animal hypertensive states there are signs that immune malfunction is present."

To support their hypothesis, the researchers manipulated the immune system of genetically hypertensive rats (SHR) to reduce the animals' blood pressure. Dr. Strausser's group is investigating the effects of a calf thymic extract called thymosin fraction 5 on immune function and blood pressure in SHR and normal Wistar-Kyoto (WKY) rats. Assisted by her former student Dr. Adrianne Bendich, who is now an immunologist at Hoffmann-La Roche, Inc., Dr. Strausser initiated trials in which the SHR's were compared with the WKY rats, which served as controls. SHR's differed from the controls in that they began to develop hypertension spontaneously a few months after birth.

The course of the disease is similar to the human pattern. Blood pressure is higher in males than in females; kidney and cardiovascular problems persist until death occurs around 15 months of age, compared to 2½ to 3 years for a normal rat. Kidney disease in SHR's appeared to be caused by the deposition of immune complexes in the glomeruli, according to Dr. Strausser.

"Administration of thymosin caused a blood pressure drop in both males and females, and within 2 to 3 weeks the pressures approached the levels of the WKY group," she says. "On termination of treatment the blood pressures remained at normal levels for several months or until the animals were stressed. By contrast, the WKY rats were unaffected by the extract, maintaining steady pressures around a norm of 120 mm Hg."

Dr. Helen R. Strausser, physiologist at Rutgers University: ***"Genetic factors may be linked to the immune response, since in both human and animal hypertensive states there are signs that immune malfunction is present."***

Immune function tests conducted on the rat spleens before and after treatment indicated that SHR's lack T suppressor cells or suppressor substances as compared to the WKY animals. "Thymosin appears to increase the number or function of these suppressor cells or substances," says Dr. Strausser. "This increase in T suppression may inhibit the formation of antibodies, causing an autoimmune-like disease. Administration of thymosin prevents the immune complex accumulation in the kidneys and consequent damage to the vascular system."

Older SHR and lupus mouse models have been shown to lack sufficient amounts of prostaglandin E, a substance that would also inhibit excess antibody production if present in sufficient amounts, Dr. Strausser says. Stimulation of prostaglandin production is currently being attempted in her laboratory in animals suffering from these autoimmune-like diseases.

As further evidence of a link between hypertension and the immune system, Dr. Strausser lowered the high blood pressures of SHR's to normal with antithymocyte serum, possibly by decreasing T helper cells, she says. The decrease in T helper cells would prevent excess antibody formation.

"Most current clinical research on hypertension focuses on physiological and hormonal effects on arterial receptors," Dr. Strausser says. "However, there may be other, more direct ways to intervene therapeutically when pressure rises. Alternating levels of immune responses and increasing the production of certain prostaglandins offer additional approaches that should be investigated."

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Dr. Strausser's research into hypertension and the immune system was funded by the National Institute on Aging, the Guggenheim Foundation, and Hoffmann-LaRoche, Inc. The research began as a pilot project of the Biomedical Research Support Grant Program of the NIH Division of Research Resources.

Stress and the Immune System: Examining the Role of Hormones

For nearly 50 years, endocrinologists have studied the hormones released from the adrenal cortex in response to stress. Recently, however, immunologists have begun to explore the various effects of these hormones on the immune system. One such stress-related effect, as demonstrated through antigenic competition, is under investigation by a New Mexico immunologist.

"It is almost a cliche to state that stress either aggravates or contributes to the origin of many illnesses, whether oncologic, bacterial, or virus-induced," says Dr. Sei Tokuda, an immunologist at the University of New Mexico Medical School. "But the investigator faces a difficult problem in determining a baseline or norm against which these phenomena can be measured. For example, the blood sampling required to study cell-mediated immune defenses is itself a stressor.

"We have approached this problem through the study of sequential antigenic competition, the suppression of an immune response by the later administration of a second, unrelated antigen. Since this involves two separate inoculations, we can compare the effect of one with the effect of another, avoiding the complications of additional stress related factors."

Antigenic competition has been studied by immunologists since the turn of the century. Early studies were clinically oriented, focusing on the response to different vaccines and the schedule of their administration. More recently, basic researchers have tried to formulate an overall theory explaining the process. Work recently performed in the New Mexico laboratory supports the theory that antigenic competition is at least partially related to stress as reflected in levels of corticosteroids, hormones secreted by the adrenal cortex.

A primary response to stress occurs when the pituitary gland secretes adrenocorticotropic hormone (ACTH), causing the adrenal glands to release corticosteroids. These hormones enter the bloodstream and increase as much as 20-fold over prestress levels. When stress is relieved, these hormonal concentrations decline, influenced by a feedback mechanism in which the hypothalamus and pituitary gland act jointly to reduce their secretion.

The action of corticosteroids in suppressing immune response is well known. It has also been shown that in stressed mice the release of ACTH lowers immune response. The influence of these hormonal pathways in controlling immune response in a living animal, however, has not been studied in depth.

In a recent set of trials at New Mexico, the adrenal glands were removed from one group of 7-week-old mice. The pituitary glands were removed from another group of the same age. Those without adrenal glands showed no significant difference in the number of cells producing antibody against sequential immunizations of horse red blood cells (HRBC) and sheep red blood cells (SRBC) spaced 3 days apart. This contrasted with the sham-operated control group, which showed a suppressed response to the second antigen. The same pattern prevailed in mice without pituitary glands.

"Our explanation is that immunization with a large dose of antigen can be viewed as a stressful situation resulting in a release of ACTH from the pituitary gland," Dr. Tokuda says. "These mice were caged separately to reduce stress and noise and were not immunized until 2 to 3 weeks after their glands had been removed. ACTH stimulates the adrenal glands to release corticosteroids, which in turn results in immunosuppression—and this resulted in the loss of immune responsiveness to the second antigen."

Removal of these glands reduced response against only SRBC in another series of trials. In addition, mature mice older than 8 months do not express sequential antigenic competition. Thus, other factors may be involved, he says, such as a loss of growth hormone, thyroid-stimulating hormone, aldosterone, or norepinephrine. The research group is currently trying to determine precise levels of hormonal regulation in these processes as well as the target cell population affected by the corticosteroids.

"Research with humans has pointed to a relationship among lowered immune response, increased epinephrine production, decreased immunoglobulin in saliva, psychological stress, and increased respiratory infection," he concludes. "The immune systems of mice and humans differ, as do their responses to stress. But it is increasingly clear that we cannot consider regulation of the immune response solely as a property of the immune system itself, but rather as linked to the nervous and endocrine systems."

Research on antigenic competition and immune response is supported by the National Cancer Institute and the Biomedical Research Support Grant Program of the NIH Division of Research Resources.

Information Exchange

Research Highlights

Short-term paralysis resulting from the interaction of marijuana with reserpine, a widely used antihypertensive drug, was observed recently in animal experiments performed by researchers at the University of Texas in El Paso (UTEP).

Reserpine in large doses induces a neurologic effect in rats that was increased more than 20-fold by delta-9-tetrahydrocannabinol (THC), one of many psychoactive compounds in marijuana. The study was conducted by Dr. Donald E. Moss and colleagues of the psychology department at UTEP. Dr. Moss believes that Parkinson's disease patients and those receiving reserpine for hypertension or other clinical purposes should be counseled to avoid the use of marijuana until the clinical significance of the interaction can be determined.

Muscular rigidity and a state of motionlessness called akinesia result from large doses of reserpine. Rats treated with the drug and placed with their forepaws on a metal bar 9 centimeters above the floor will remain motionless in that position for relatively long periods of time. Untreated control animals, on the other hand, quickly resume normal posture and activity. Since THC also produces an akinetic-like state under certain circumstances, Dr. Moss attempted to determine if it would interact with reserpine to a degree measurable by the bar test.

In the first experiment, rats treated with reserpine alone were motionless with forepaws on the bar for a little more than half a minute. Akinesia was increased to an average of more than 15 minutes when reserpine was followed by THC. Untreated control animals, as well as those given THC only, had no akinetic signs and were off the bar within 1 to 3 seconds. The reserpine dosage was 7.5 milligrams per kilogram of body weight (mg/kg BW) injected intraperitoneally 24 hours before the bar test. THC was given orally at a level of 10 mg/kg BW at 5 hours prior to testing.

"Many reserpine/THC-treated animals remained motionless much longer than the arbitrary 30-minute maximum allowed for the test," Dr. Moss says, "and bar times of longer than 1 hour have been observed."

Further experimentation revealed that the enhancing effect of THC on reserpine-induced akinesia occurred within the first hour after oral administration and was not completely dissipated until about 11 hours later. "This corresponds very closely with the time course of the psychoactive effects of marijuana in humans who take the drug orally," Dr. Moss says.

He believes that the pronounced behavioral effect of THC on reserpine-induced akinesia, which is reproducible and easily measured, may provide a key to measuring THC's action on other drugs that have important effects on nervous system function. "This is of particular interest," he says, "because there is grave concern today about possibly profound and unpredictable interactions of marijuana with other commonly used drugs.

"On the brighter side," says Dr. Moss, "the most important and direct application of our findings may be the use of THC, or a similar drug without psychoactive effects, to increase the efficacy of reserpine in the treatment of

Rat treated with THC and reserpine: ***THC increases the akinetic state induced by reserpine by more than twentyfold.***

Photo courtesy of Instructional Technology Center, UTEP

Dr. Donald E. Moss, principal investigator (left), and Mr. Nino Gonzalez, Minority High School Student Research Apprentice Program: ***weighing rats used in a study of the interaction between reserpine and THC, one of the psychoactive compounds in marijuana.***

Photo courtesy of Instructional Technology Center, UTEP hyperkinetic motor disorders. Reserpine, because of its inhibiting effect on the motor system, was one of the first agents used successfully in the treatment of various choreiform diseases and has been of benefit in tardive dyskinesia and in the treatment of Huntington's disease."

Since reserpine produces a syndrome very similar to parkinsonism, it may be used to provide an animal model of the disease. Dr. Moss used the rat model in an attempt to learn something about the mechanism of THC's potentiation of reserpine-induced akinesia. An interesting finding was that ethopropazine (Parsidol), an anticholinergic drug used in treating Parkinson's disease, completely blocked the effect of THC on reserpine in the model. He reports that tests of other drugs, which have no known important effects on the extrapyramidal motor system, showed no influence on the THC-reserpine interaction.

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Research on the interaction of marijuana and reserpine is supported by the National Institute on Drug Abuse, the National Institute of Neurological and Communicative Disorders and Stroke, the Minority Biomedical Support Program of the NIH Division of Research Resources, and a gift from the Moss family.

A hypothetical model linking pancreatic cancer with metabolic imbalances, lifestyle, and diet has been developed by two researchers at the Georgetown University School of Medicine.

According to the model, smoking, alcohol consumption, and eating high-fat foods upset the balance between proteolytic enzymes and their inhibitors. This imbalance is present during pancreatitis, an inflammation of the pancreas that may be a precursor to pancreatic cancer.

Proteolytic enzymes, usually called proteases, are components of the pancreatic juice secreted into the small intestine for the purpose of digesting proteins. Their action is limited by protease inhibitors, which prevent these enzymes from consuming the body's own tissues.

"An enzyme inhibitor of particular importance is alpha-1-antiprotease (A_1-AP), which suppresses the proteolytic activity of digestive enzymes such as elastase and trypsin," says Dr. Gregory Morosco of Georgetown University. "This inhibitor is manufactured in the liver, possibly also in the pancreas itself, and combines with a protease to form an inactive complex."

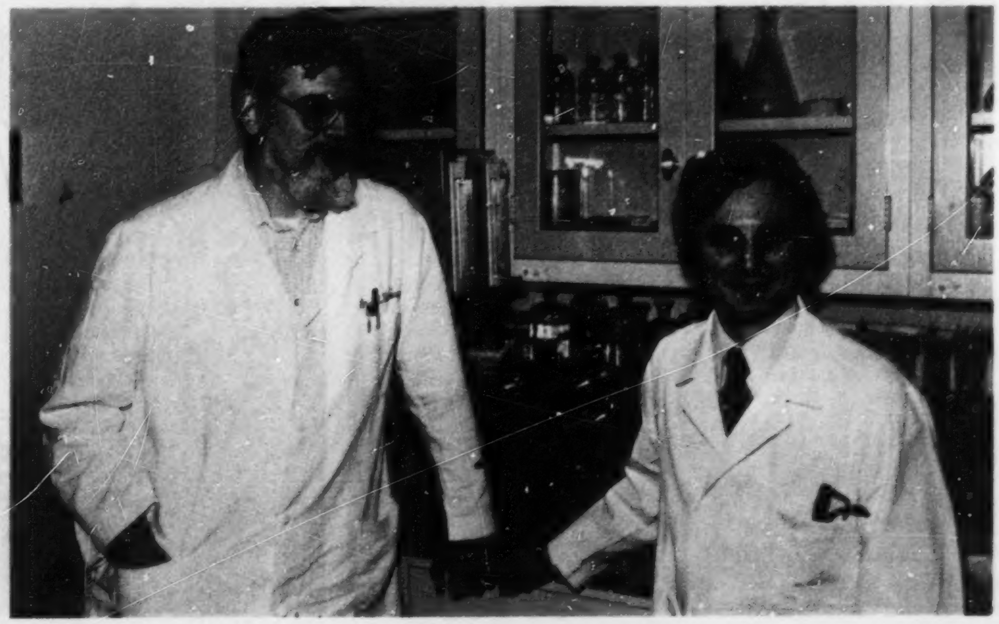
Together with Dr. Gerald C. Goeringer, Dr. Morosco has developed the hypothesis that suppression of this inhibitor or premature activation of proteases, or both, may result in an enzyme imbalance leading to pancreatitis and possibly pancreatic cancer. Genetic factors may also be involved, because varying levels of A_1-AP have been related to heredity in humans.

The researchers cite the findings of Dr. Walter Troll at New York University School of Medicine, which indicate that some proteases can promote tumor growth in mice while the inhibitors can suppress growth of the same tumors. Tumor cells also frequently show higher levels of proteases than normal cells. Pancreatitis can be experimentally induced in the laboratory by elastase, a protease found in both humans and animals.

Pancreatic cancer was responsible for 27,777 deaths in the United States in 1978, according to the National Center for Health Statistics. Prognosis for pancreatic cancer is the poorest of any major variety of cancer; it is nearly always fatal.

Doctors believe that eating habits and lifestyle may cause the metabolic imbalances that appear to influence the development of this cancer. "Cigarette smoking, high-fat diet, and alcohol have all been implicated in several epidemiologic investigations," Dr. Morosco says. "But laboratory work with animals that could shed light on the possible tumorigenic action of these life-

Drs. Gerald C. Goeringer (left) and Gregory Morosco of Georgetown University: *linking lifestyle to the occurrence of pancreatic cancer.*



style factors is sadly lacking despite such excellent leads. In our own work, Dr. Goeringer and I have concentrated on showing that these enzymatic imbalances are linked to lifestyle changes."

The two investigators conducted experiments in beagle dogs that had been exposed to smoke for 600 days from either high- or low-nicotine cigarettes. Tracheostomies were performed so that freshly generated puffs of smoke could be administered into the respiratory system. Pancreatic elastase levels increased significantly with high-nicotine cigarettes but much less with low-nicotine cigarettes. Protease inhibitor levels were significantly decreased during exposure to high-nicotine smoke as well.

"Other research suggests that nicotine alone does not suppress A_1-AP," Dr. Goeringer says. "But nicotine is metabolized by the body into a variety of different compounds such as nitrosamines. It is of interest that nitrosamines injected into test animals can produce pancreatic tumors similar to those found in humans. Recent evidence from other laboratories as well as our own indicates that the inhibitory capacity of protease inhibitors can be severely compromised in both animals and humans after exposure to cigarette smoke."

The researchers also found that significant increases in elastase activity occurred in beagle dogs when levels of blood nicotine reached 50 to 70 nanograms per milliliter. Exposure to nicotine-free cigarettes did not result in the secretion of active elastase. "The appearance of these enzymes is of interest," Dr. Morosco says, "because they have been implicated in causing lung and cardiovascular injury as well as pancreatic injury. Thus, they are prime candidates as biological indicators of adverse response to chemical exposures."

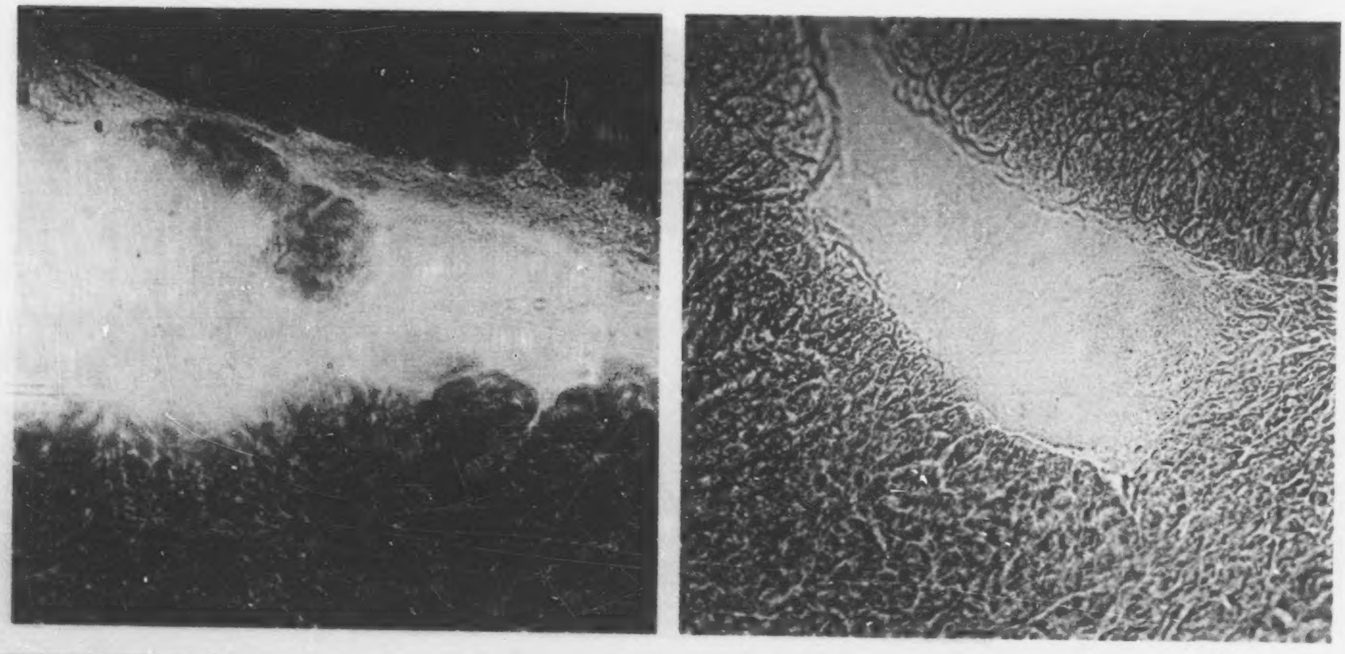
Epidemiologic evidence gathered from countries around the world indicates a high correlation between mortality rates from pancreatic cancer and fat consumption. Pancreatic tissue taken from baboons who have consumed a high-fat diet at the Southwest Research Institute in San Antonio showed an increase in protease activity, as compared to a control group, the researchers say.

"Other lifestyle factors cited by epidemiologists are alcohol and coffee," Dr. Morosco says. "Alcohol consumption has been positively associated in some but not all studies. Further investigations are needed. Chronic alcohol exposure does increase protease secretion, however, and substantial evidence exists indicating that alcoholics are far more likely to develop pancreatitis than the general population."

Coffee drinking has also aroused interest; for example, the widely publicized Harvard study that supports the findings of a 1970 report showing a significant relationship between coffee consumption and pancreatic cancer mortality among males in 20 countries. This is another area in which laboratory work with animals is needed, says Dr. Morosco. He and Dr. Goeringer propose that the effect of coffee, alcohol, smoking, and high-fat diets be tested with an appropriate blend of epidemiologic and experimental studies. "Much more data are needed to make stronger connections between lifestyle factors and pancreatic cancer," says Dr. Morosco.

Additional reading:

1. Morosco, G. J., and Goeringer, G. C., Lifestyle factors and cancer of the pancreas: a hypothetical mechanism. *Medical Hypotheses* 6:971-985, 1980.
2. Morosco, G. J., and Goeringer,



Pancreatic cells along ductal lumen in the baboon on high-fat diet (left) and regular chow: *high-fat foods increase the activity of proteolytic enzymes, producing conditions that lead to pancreatitis, a possible precursor of pancreatic cancer.*

G. C., Pancreatic elastase and serum alpha-antitrypsin levels in beagle dogs smoking high- and low-nicotine cigarettes: possible mechanism of pancreatic cancer in cigarette smokers. *Journal of Toxicology and Environmental Health* 5:879-890, 1979.

3. Troll, W., Blocking tumor promotion by protease inhibitors. In *Fundamentals in Cancer Prevention*, Tokyo, University of Tokyo Press, 1976, pp. 41-55.

4. Troll, W., Klassen, A., and Janoff, A., Tumorigenesis in mouse skin: inhibition by synthetic inhibitors of proteases. *Science* 169:1211, 1970.

5. Morosco, G. J., Nightingale, T. E., Frasinel, C., and Goeringer, G. C., Pancreatic elastase activation as a possible indicator of the relative hazard of different cigarettes. *Journal of Toxicology and Environmental Health* 8:89-94, 1981.

Research into factors associated with pancreatic cancer has been supported by grants from the National Cancer Institute and the Biomedical Research Support Grant Program of the NIH Division of Research Resources.

Research Service

Bullfrog Culture System

A limited number of live high-quality research bullfrogs (*Rana catesbeiana*) are available on occasion to researchers on an advance-order basis from a resource maintained at Louisiana State University (LSU) in Baton Rouge and supported by the Animal Resources Program of the NIH Division of Research Resources. Certain tissue cultures may occasionally be obtained by arrangement with either of the coprincipal investigators, Dr. Dudley D. Culley, Jr., professor of fisheries, or Dr. Robert L. Amborski, professor of microbiology.

Consultation on design and operation of amphibian resources, information on care and management of *R. catesbeiana*, and a graduate student training program are other services of the facility. Dr. Amborski also provides, for a fee, consultation and, occasionally, laboratory diagnostic services on bullfrog diseases. A vaccine is being developed for a major pathogenic bacterium (*Aeromonas hydrophila*) of frogs and is expected to be ready for testing in about 6 months. Techniques for mass culture of the bacterium and for, toxin extraction can be obtained from Dr. Amborski.

The Louisiana resource can also serve as a contact point with a large Brazilian bullfrog culture system that will be able to supply very large quantities of bullfrog organs and tissues to the research community.

Four lines of *R. catesbeiana*—laboratory-bred, laboratory-raised, wild-caught and laboratory-conditioned, and wild-caught bullfrogs—are available from the LSU resource, when the laboratory has excess frogs.

Wild-caught and laboratory-conditioned bullfrogs are collected or purchased from a biological supply house and maintained at the facility for 30 to 60 days under strict laboratory conditions.

Laboratory-raised bullfrogs are raised from eggs or larvae (tadpoles) collected in the wild, carried through metamorphosis in the laboratory, and then maintained for the period of time necessary for the project in which the animals are to be used.

Laboratory-bred bullfrogs are reproduced from eggs or tadpoles from the resource's parent stock, which is maintained in the laboratory under defined conditions.

Wild-caught bullfrogs are provided only by special request.

Usually researchers must plan well in advance for live frogs or tissue cultures from the LSU resource. Special processing or handling requirements for the frogs and tissue cultures must be communicated to the resource at an early date.

The resource provides mainly young, laboratory-raised bullfrogs. Older animals are provided on special request and are usually wild-caught and laboratory-conditioned bullfrogs. Tadpoles, when available, can be obtained from hatching up to about 12 weeks of age. Juveniles can be provided at any stage from metamorphosis up to 6 months of age.

Small quantities of tissues and organs, when available, can be obtained by special arrangement.

Prices for live frogs range from $2 to $15 per animal, depending mainly on the extent of maintenance and care. Prices of organs and tissues are "extremely variable and negotiable," says Dr. Culley.

Live bullfrogs are shipped in plastic-coated or wax-coated boxes packed with moistened synthetic foam material to protect and insulate the animals. Delivery by plane to any point in the United States is usually possible within 12 hours. Dr. Culley says they rarely lose an animal in shipment.

The LSU resource has supplied bullfrogs to a number of biomedical centers around the country for a variety of research projects. The National Aeronautics and Space Administration (NASA), for example, used bullfrogs from the resource in a study designed to determine the cause of sickness experienced by astronauts in long flights. The disorder was thought to be related to the inner ear, and large bullfrogs were sent into space as part of their effort to determine the exact cause of the sickness.

The cause of muscle degeneration in the tadpole tail as it goes through metamorphosis was investigated at Tulane University Medical School. Animals are now being supplied to Harvard University Medical School for an ongoing project on nerve regeneration.

The LSU staff has developed a very good diet for the tadpole and has supplied it to many scientists around the country. It can be obtained from the resource in premix powder form for $3 per pound.

The resource also supplies researchers with information on many aspects of reproduction, nutrition, maintenance, and disease control with respect to *R. catesbeiana*. A bibliography of all resource publications is available upon request. Copies of the individual articles are provided free. Researchers are encouraged to contact the resource for details not covered in the publications.

The resource is also involved in the graduate education program of LSU. The program operates in four laboratories. The culture facility is located at the Fisheries Research Station, a 200-acre facility with laboratories, hatcheries, and more than 150 ponds. A large-scale aquaculture program is carried on, in which students get exposure to the culture, management, nutritional requirements, and diseases of the bullfrog and a variety of other aquatic animals cultured under different conditions.

Frogs have a long history in biomedical research and constitute most of the amphibians used each year in teaching and research, according to Dr. Culley. He points out that *R. catesbeiana*, like other frogs, has a set of characteristics that make them good animals for research. For example, their systems are very similar to the systems of other vertebrates. Eggs are available in large quantities for ovulation, reproduction, and developmental studies. They are transitory between aquatic and terrestrial life, reasonable in size, ecothermic, and accessible to direct examination through all developmental periods. The frog is a "naked vertebrate" and thus is well-suited for studies on skin grafting and related research. Frogs are also inexpensive to maintain. The facilities are very simple compared to the expensive types required for mammals and birds. Moreover, frogs do not require the care and attention necessary for mammals.

Investigators interested in tissues and organs are advised to make contacts now. However, only large orders involving several thousand or more specimens will be considered. Orders and specific instructions for handling the specimens should be placed at least 6 months before the material is needed.

The Brazilian firm is also considering providing live bullfrogs for the United States food market and for biomedical research and educational purposes. Drs. Amborski and Culley believe that the well-controlled culture process should yield very high-quality animals.

For further information about the availability of frogs, tadpoles, and food, contact:

Dr. Dudley D. Culley, Jr.
249 Agricultural Center
Louisiana State University
Baton Rouge, Louisiana 70803
Telephone (504) 388-6051.

Inquiries regarding disease-related problems and tissue culture should be directed to:

Dr. Robert L. Amborski
Department of Microbiology
Louisiana State University
Baton Rouge, Louisiana 70803
Telephone (504) 388-2601.

Dr. Dudley D. Culley, Jr., in Louisiana bullfrog resource: *providing animals and information to biomedical centers across the country.*

Note on CLINFO

On page 10 of the December 1981 issue of the *Research Resources Reporter*, a specialized minicomputer system called CLINFO is described as eliminating the need to hire statisticians or to have a processing facility for data analysis. In fact, the policy of the General Clinical Research Centers (GCRC) Program, which funds the CLINFO project, is to "strongly encourage consultation with experts in statistics in conjunction with the use of CLINFO," says Dr. Wiliam DeCesare, director of the GCRC Program.

Photo by Paul Brown, Valley Times

Dr. B. Vaughn Critchlow, new director of the Oregon Regional Primate Research Center as of May 1, 1982.

Director Named for Oregon Primate Center

Dr. B. Vaughn Critchlow, an internationally recognized expert on interaction between the brain and the endocrine system, has been named director of the Oregon Regional Primate Research Center effective May 1. He will replace Dr. William Montagna, who recently retired after serving as center director since 1963. The center is one of the seven primate research centers supported by the NIH Division of Research Resources. A major focus of research at the center concerns reproductive biology.

Dr. Critchlow has been professor and chairman of the department of anatomy at the Oregon Health Sciences University (OHSU) since 1972. He received his bachelor's degree from Occidental College in Los Angeles and his doctoral degree from the University of California at Los Angeles. He then joined Baylor University College of Medicine in Houston, where he rose to the rank of professor and acting chairman of the anatomy department. While at Baylor he was visiting investigator at the Nobel Institute of Neurophysiology, Karolinska Institute, in Stockholm. He has received several awards for excellent teaching at Baylor and the OHSU.

In addition to his duties as center director, Dr. Critchlow plans to continue his research on how the brain controls growth hormone. He will also pursue research on how stress affects the secretion of hormones in the body. To accomplish this, he is investigating the brain structures that influence the pituitary, or "master" gland, which releases hormones that stimulate specific body organs involved in the stress reaction.

Comparative Pathology Directory Published

A new guide to collections of biological materials and information useful in comparing various disease processes has been published. Entitled *Directory: Resources of Biomedical and Zoological Specimens*, the 53-page booklet briefly describes the contents of 114 collections, located worldwide, and lists the contact point for each collection.

Published by the Registry of Comparative Pathology, Armed Forces Institute of Pathology, which is supported in part by the NIH Division of Research Resources, the publication is intended as a resource guide for scientists. Entries in the directory are listed according to the type of the collection.

The contents of the directory are separated into five sections: general collections (material from many diseases and conditions and from many species); specific collections (material of a specific nature or from one animal category); museum collections (preserved specimens, skulls, skins, skeletons); miscellaneous collections; and catalogs and guides. The largest section, specific collections, which contains 75 citations, includes subsections on aquatic animals, bones and teeth, laboratory animals, neuropathology, nonhuman primates, parasites, reproduction, tumors, and zoo and wild animals. Subsections are cross-referenced to other pertinent citations in the book.

A single free copy of the new publication may be had by writing to the Registry of Comparative Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306.

Comparative Pathology Course Announced

The 9th annual comparative pathology course will be presented from May 3 to 5, 1982, at the Armed Forces Institute of Pathology, Washington, D.C. This course is designed to bring attention to disease processes in animals for which a similar entity occurs in man. Differences and similarities of pathologic lesions, as well as the biological behavior of specific entities will be compared in animals and man.

Military and federal service employees in the medical and veterinary fields are requested to consult respective agency regulations for appropriate application procedures. Civilian physicians, veterinarians, and allied scientists are invited to apply and will be considered on a space available basis.

Application forms may be obtained by contacting the Director, Armed Forces Institute of Pathology, Washington, D.C. 20306, telephone (202) 576-2452. Completed application forms should be returned by April 5, 1982. Those who are not American citizens must submit a $75 fee payable to the Treasurer of the United States.

Meetings

Computer Symposium

he Sixth Annual Symposium on
Computer Applications in Medi-
al Care will be held in Washing-
on, D.C., from October 30 to
lovember 2, 1982. The sympo-
ium is designed to inform physi-
ians, biomedical scientists,
ngineers, health care admin-
trators, health care profes-
ionals, and information
chnology specialists about cur-
ent and potential applications of
omputers in health care. More
an 2,000 persons are expected
attend. For more information,
ontact:

r. Bruce I. Blum
CAMC—Office of CME
00 K Street, NW
ashington, D.C. 20037
lephone (202) 676-4285

onference on Engineering Medicine and Biology

e 35th Annual Conference on
gineering in Medicine and
ology will be held in Phila-
lphia, Pennsylvania, from Sep-
nber 22 to 24, 1982. In addition
the usual topics, special ses-
ns will be presented on bio-
terials, bioelectric phenom-
a, biorheology, heat and mass
nsfer, sensory communications, hemodynamics, respiratory mechanics, electrophysiology, radiology and imaging, biosolid mechanics, instrumentation and controls, and orthopedics and implants.

Deadline for receipt of abstracts is April 15, 1982. For further information, contact:

Patricia I. Horner
4405 East-West Highway
Bethesda, Maryland 20814
Telephone (301) 657-4142

Biotechnology Resources Program Hosts Colloquium

A "PROPHET Users Colloquium" will be held in Airlie, Virginia, from May 12 to 14, 1982. The colloquium is by invitation only and will address the emerging scientific needs of the 700 biomedical researchers using the PROPHET computer network at 40 sites around the country. The colloquium is expected to generate a list of new statistical and analytical tools to be developed to meet emerging research needs. For further information contact:

Dr. Jack Hahn
Head, Computer Technology
Section
Biotechnology Resources
Program
Division of Research
Resources
Building 31, Room 5B43
Bethesda, Maryland 20205
Telephone (301) 496-5411

Symposium on Biostereometrics

"Biostereometrics '82", the third in a series of meetings sponsored by the International Society for Optical Engineering, will be held in conjunction with the 26th Annual International Technical Symposium and Instrument Display and the 15th International Congress on High-Speed Photography and Photonics in San Diego, California, from August 23 to 27, 1982. An equipment and poster exhibit will be held in conjunction with the symposium.

The program will focus on the three-dimensional quantification of biological form and function over time and will include research presentations on the historical background, mathematical foundations, data acquisitions, data analysis and displays, and clinical and nonclinical applications of biostereometrics.

For further information write or call: SPIE, P.O. Box 10, Bellingham, Washington 98227, telephone (206) 676-3290.

March 7-12, 1982

Title: *Chemistry and Biology of Interferons: Relationship to Therapeutics*
Location: *Squaw Valley, California*
Contact: *UCLA Symposia*
Molecular Biology Institute
Los Angeles, California 90024
Telephone (213) 206-6292

March 25-27, 1982

Title: *National Pediatric Infectious Disease Seminar*
Location: *Las Vegas, Nevada*
Contact: *J. D. Nelson, M.D., or*
G. H. McCracken, M.D.
Department of Pediatrics
University of Texas Health Science Center at Dallas
5323 Harry Hines Boulevard
Dallas, Texas 75235
Telephone (214) 688-3391

March 25-27, 1982

Title: *National Conference on Advances in Infant Nutrition and Pediatric Gastroenterology*
Location: *Sarasota, Florida*
Contact: *Rayna Dutton*
Coordinator
Continuing Medical Education
Children's Hospital
219 Byrant Street
Buffalo, New York 14222
Telephone (716) 878-7630

March 26-27, 1982

Title: *Recent Research Advances in Aging, Heart Disease, Cancer, and Other Age-Related Disorders*
Location: *San Diego, California*
Contact: *Robert J. Morin, M.D.*
President
American Longevity Association
Harbor-UCLA Medical Center
Torrance, California 90509
Telephone (213) 533-2233

April 12-15, 1982

Title: *Symposium on Genetic Mechanisms of Carcinogenesis*
Location: *Oak Ridge, Tennessee*
Contact: *C. W. Yang*
Oak Ridge National Laboratories
Building 9211, MS 001
P.O. Box X
Oak Ridge, Tennessee 37830
Telephone (615) 574-0700

April 14-16, 1982

Title: *Cochlear Implantation in Sensorineural Hearing Loss*
Location: *New York, New York*
Contact: *Conference Department*
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021
Telephone (212) 838-0230

April 15-23, 1982

Title: *66th Annual FASEB Meeting*
Location: *New Orleans, Louisiana*
Contact: *Office of Scientific Meetings*
FASEB
9650 Rockville Pike
Bethesda, Maryland 20814
Telephone (301) 530-7010

April 18-23, 1982

Title: *Rational Basis for Chemotherapy*
Location: *Keystone, Colorado*
Contact: *UCLA Symposia*
Molecular Biology Institute
Los Angeles, California 90024
Telephone (213) 206-6292

April 21-23, 1982

Title: *5th Intraocular Lens Symposium*
Location: *Los Angeles, California*
Contact: *American Intraocular Implant Society*
P.O. Box 3140
Santa Monica, California 90403
Telephone (213) 395-3937

May 14, 1982

Title: *23rd Annual Meeting of the Cystic Fibrosis Foundation*
Location: *Washington, D.C.*
Contact: *R. J. Beall, Ph.D.*
6000 Executive Boulevard
Suite 309
Rockville, Maryland 20852
Telephone (301) 881-9130

May 17-19, 1982

Title: *Workshop on Time-of-Flight Emission Tomography (By invitation only)*
Location: *St. Louis, Missouri*
Contact: *Dr. Jack Hahn*
Head, Computer Technology Section
Biotechnology Resources Program
Division of Research Resources
Building 31, Room 5B43
Bethesda, Maryland 20205
Telephone (301) 496-5411

October 4-10, 1982

Title: *First Australian Congress on Medical Climatology: Bio-Psycho-Physiological Effects of Small Air Ions*
Location: *Sydney, Australia*
Contact: *Dr. Charles Wallach*
Executive Director
International Bio-Environmental Foundation
P.O. Box 994
Silver Spring, Maryland 20910
Telephone (301) 585-8881

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Research Resources
Reporter

April 1982, Volume VI, No. 4

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

Serving Animal Resources,
Biotechnology Resources,
General Clinical Research Centers,
Minority Biomedical Research Support,
and Biomedical Research Support

6/4

This Issue:

Ten Years of MBRS Program
Tranquilizer Dependence
Advances in Neuroendocrinology
STEM Use of Heavy Atoms
Miniature Electronic Sensors

by
Gail Shiner Makulowich

Primate Studies Focus On Dietary Fats

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Although the relationship between dietary fats and the risk of coronary heart disease (CHD) has been studied extensively, the best ratio of dietary polyunsaturated fats to saturated fats (P/S ratio) and the healthy amount of total dietary fat are still being debated. These issues are being investigated in two ongoing studies at the New England Regional Primate Research Center.

One 3-year study nearing completion is focused on dietary regulation of high-density lipoprotein (HDL) and its alleged protective role against CHD. A recently initiated 5-year study is investigating the validity of the dietary goals published by the Senate Select Committee on Nutrition and Human Needs in 1977. These goals, which specify reduced intakes of certain types of fat, total dietary fat, and dietary salt to prevent hyperlipidemia and hypertension, provided the basis for the general dietary guidelines published jointly by the U.S. Department of Agriculture (USDA) and the Department of Health and Human Servi-

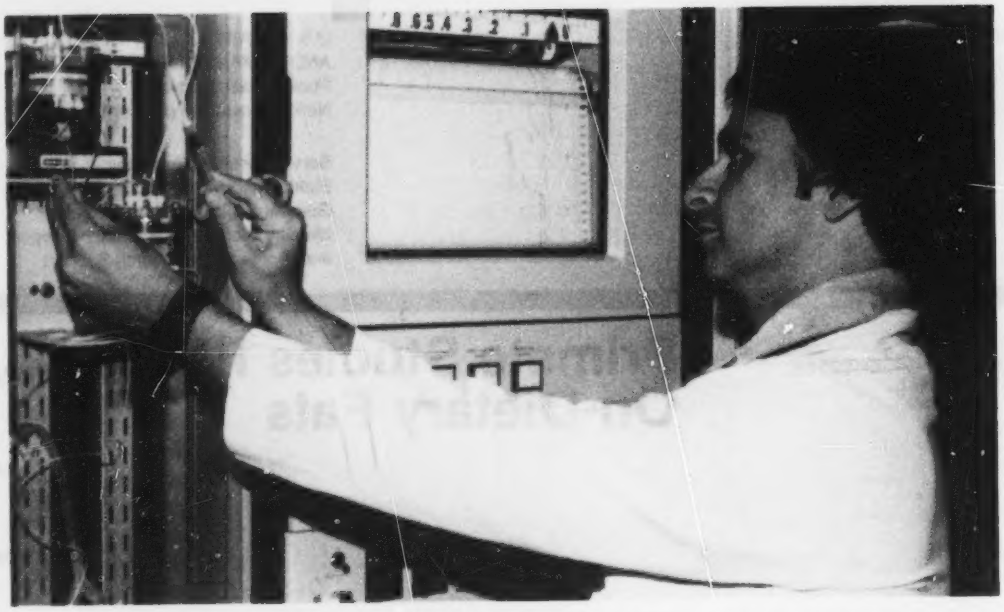
Cynomolgus monkeys: ***models for comparing the health effects of the average American diet with effects of the diet recommended in 1977 by the Senate Select Committee on Nutrition and Human Needs.***

HE 20. 3013/6:

DIETARY FATS *continued*

ces (DHHS) in 1980.

Research has established that the consumption of excessive saturated fat or cholesterol can result in a high level of blood cholesterol, which is associated with increased risk of coronary heart disease. The particular lipoprotein class that transports circulating cholesterol is another important risk indicator of CHD. A number of researchers have labeled high-density lipoprotein (HDL) the "good" lipoprotein by virtue of its ability to remove cholesterol from cells in tissue culture and its strong negative correlation with CHD incidence. They have labeled low-density lipoprotein (LDL) the "bad" lipoprotein, because it has been found to increase the content of cellular cholesterol in tissue culture and it is correlated with the incidence of CHD.



HDL is a plasma protein containing more protein and less cholesterol and triglycerides than LDL, which is also a plasma protein. These researchers currently believe that HDL exerts a protective effect against heart disease for two reasons. It is thought to remove cholesterol from cells of peripheral tissue such as blood vessel walls for subsequent transport to the liver, where it is broken down. Secondly, HDL is thought to compete with and therefore prevent the uptake of the supposedly atherogenic LDL by specific cell receptors.

However, findings by Dr. Robert J. Nicolosi, assistant professor of pathology at the Harvard Medical School and chairman of the nutrition division at the New England Regional Primate Research Center, and his colleagues join the findings of other studies which question the growing belief that high levels of HDL may protect against heart disease. To study how diet can influence circulating levels of HDL and other lipoproteins, Dr. Nicolosi's group fed primates a polyunsaturated fat (PUFA) diet of semipurified agar cakes containing corn oil with a P/S ratio of 4.5 (i.e., 4.5 parts of dietary polyunsaturated fats to 1 part of dietary saturated fats). The animals, rhesus, cebus, and squirrel monkeys, were on the diet for 3 to 7 years. The results demonstrated that the PUFA diet significantly reduced not only LDL cholesterol, but HDL cholesterol as well.

"Initially I was concerned about the reduction in HDL cholesterol levels with the highly unsaturated fat diet, since this is the lipoprotein class negatively correlated with heart disease," explains Dr. Nicolosi. "However, despite the decrease in both LDL and HDL levels in animals on the PUFA diet, biochemical, histochemical, and electron microscopical analyses of thoracic and abdominal aortas of monkeys have not only failed to demonstrate any atherosclerotic involvement but, in fact, they have consistently shown less lipid accumulation. Taken together these observations lead me to question the importance of high and low HDL levels as indicators of CHD risk."

The few studies on humans have also demonstrated significant reductions in HDL as a result of diets containing a high percentage of polyunsaturated fats, according to Dr. Nicolosi. One study on humans also indicated that decreased synthesis of HDL was associated with a PUFA diet with a P/S ratio of 4.5. These investigators were concerned about recommending a diet high in polyunsaturated fats, according to Dr. Nicolosi, because the decrease in HDL synthesis could presumably infer less HDL availability to clear cholesterol from tissues. This would indicate that consumption of a PUFA diet might not reduce the risk of heart disease after all.

However, when very similar HDL metabolic studies were carried out in monkeys fed PUFA diets containing a P/S ratio of 4.5, a decrease in HDL synthesis was not associated with the PUFA-induced reduction in HDL, according to Dr. Nicolosi. Instead, a significant increase in clearance of HDL and a tendency toward enhanced HDL synthesis were associated with the PUFA diet. "The explanation for the difference in results between the human studies and monkey investigations is not apparent, but this difference may be due to the dura-

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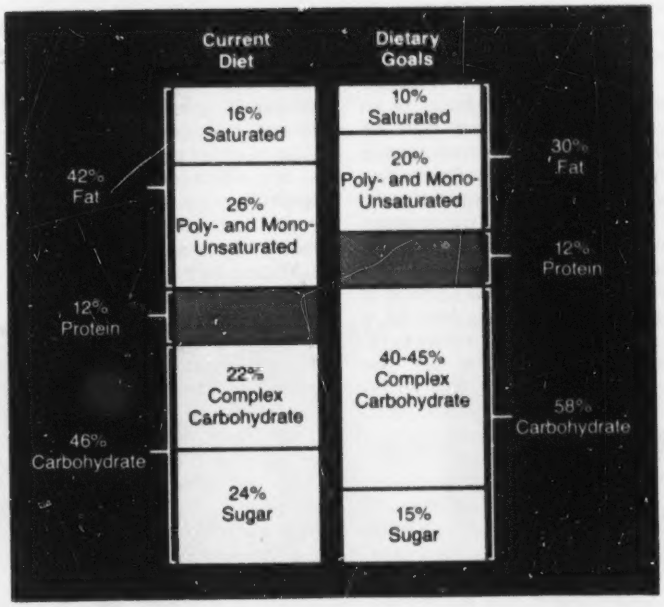
Subscription to the *Reporter* is free
upon request to the above address.

Dr. Robert J. Nicolosi, chairman of the nutrition division of the New England Regional Primate Research Center: *studying the effects of dietary fats on lipoprotein metabolism and coronary heart disease.*

Comparison of the average American diet and the recommended dietary goals.



Infant cynomolgus monkey participating in diet research: *by studying the health effects of the recommended diet from infancy to 5 years of age, researchers will be better able to assess benefits of the recommended dietary goals for humans.*

tion of dietary treatment," asserts Dr. Nicolosi. "The humans were on diets for only a few weeks, whereas the monkeys were fed PUFA diets for several years."

The mechanism of increased clearance of HDL with PUFA-feeding is unknown but may be associated with increases in certain enzymes that catabolize lipoproteins, according to Dr. Nicolosi. Two such enzymes, lipoprotein lipase and lecithin cholesterol acyltransferase, are known to have increased activity with PUFA-feeding that may be related to diet-induced changes in lipoprotein composition and size. "However, it is hard to discuss mechanisms when the true function of HDL in vivo is not known," says Dr. Nicolosi. "Except for the negative correlation with CHD derived from epidemiological studies, the alleged protective effect of HDL against heart disease is obtained from in vitro tissue culture experiments, which may not represent the true situation in the whole organism."

It is important to be cautious about data interpretation and extrapolation, he says. For example, the epidemiological data in humans indicate that the HDL subclass HDL$_2$ is more negatively correlated with risk of CHD than is HDL$_3$. Yet, investigators including Dr. Nicolosi have recently demonstrated that the atherosclerosis-resistant cebus monkey has more HDL$_3$, while the atherosclerosis-susceptible squirrel monkey has more HDL$_2$. Thus, there is still much to learn about HDL function and the biological significance of high and low HDL levels.

"It is only in the last 5 to 10 years that the role of HDL has been extensively studied, despite the fact that Dr. J. W. Goffman and colleagues showed a negative correlation between HDL and heart disease in the 1950's," comments Dr. Nicolosi. "At the time, LDL studies were in vogue, so most investigators studied LDL rather than HDL. In 1975, however, epidemiological studies by Drs. G. J. and N. E. Miller in the United Kingdom renewed interest in the hypothesis of negative correlation between HDL and heart disease." Now much research is being done to understand the role of HDL and the effect of dietary fats on these lipoproteins and heart disease.

The state of the art in studying lipoprotein metabolism has improved much in the last 3 or 4 years, according to Dr. Nicolosi. This improvement and the more sophisticated mathematical models now used for interpreting data will aid new research and currently are being used in a study of

DIETARY FATS *continued*

dietary goals begun by Dr. Nicolosi and his colleagues at the primate center last year.

In this study the investigators are comparing the effects of an average American diet with the effects of the diet recommended by the Senate Select Committee on Nutrition and Human Needs. Their subjects, 100 cynomolgus monkeys, are being fed the diet from infancy to 5 years of age. The average American diet consists of 42 percent of calories from fat, a P/S ratio of 0.5, and a daily equivalent of 12 grams of salt. The recommended diet consists of 30 percent of calories from fat, a P/S ratio of 1, and 5 grams of salt.

"The PUFA diet with a P/S ratio of 4.5 fed the rhesus, squirrel, and cebus monkeys is more extreme than the P/S ratio of the average American diet and may have been responsible for the markedly reduced levels of HDL we found in the study we just completed," suggests Dr. Nicolosi. "A more reasonable P/S ratio of 1—an equal amount of polyunsaturated and saturated fats, which is specified by the dietary goals—may not elicit such a response, although this remains to be demonstrated."

To compare the effects of the recommended diet with the average American diet, the 100 monkeys are being divided into 10 groups and fed an agar-based diet containing varied ingredients. The agar cakes contain carbohydrates such as sucrose and dextrin, a combination of animal and vegetable protein, and three different kinds of fat — butterfat, which is high in saturated fat; corn oil, which is high in polyunsaturated fat; and olive oil, which is high in monounsaturated fat—to form the level and type of fat desired. The appropriate levels of salt, minerals, and vitamins are then added. Dietary ingredients are quantitatively measured in each batch of agar to ensure uniformity of diet. Quality control of the purified diet and frequent measurements of each monkey's hematocrit and hemoglobin, electrolytes, bilirubin, and blood pressure, in addition to the usual measurement of lipoproteins, make this a noteworthy study, says Dr. Nicolosi.

"This study is unique because it will not only provide us with the opportunity to investigate the interaction of various components of the recommended dietary goals for a lengthy duration—one-fourth of the monkey's lifespan—but it will also enable us to better grasp the ramifications of implementing these dietary goals from birth," points out Dr. Nicolosi. "This is a question that could not be addressed in human studies for obvious ethical reasons."

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The study on rhesus, squirrel, and cebus monkeys was supported by the National Heart, Lung, and Blood Institute, the U.S. Department of Agriculture, Harvard School of Public Health, and the Animal Resources Program of the NIH Division of Research Resources (DRR). Study of the recommended dietary goals at the primate center is supported by Frito-Lay, Inc., R. J. Reynolds Co., and the DRR Animal Resources Program.

Squirrel monkey: ***susceptible to atherosclerosis.***

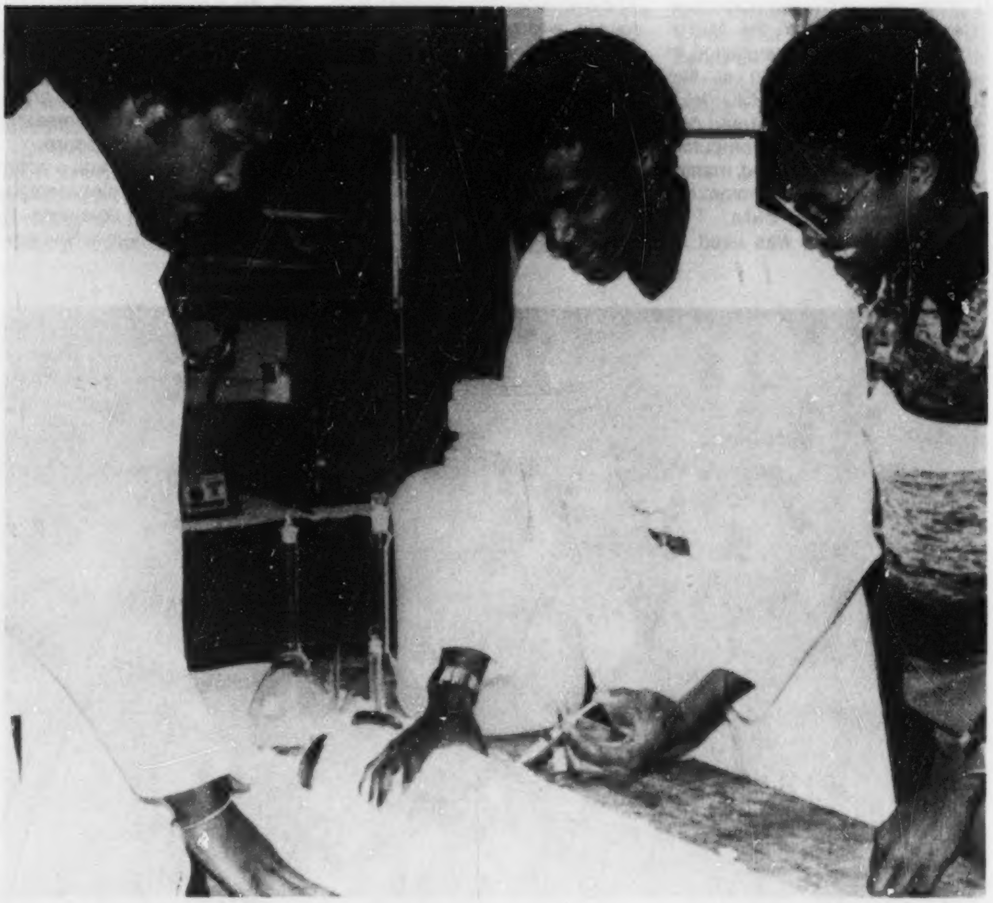
Cebus monkey: ***resistant to atherosclerosis.***

MBRS Program Celebrates Decade of Progress

"There's definitely been a quantum jump in the quality and type of biomedical research being done by minority scientists today compared to 10 years ago," says Dr. Ciriaco Gonzales, director of the DRR Minority Biomedical Research Support (MBRS) Program. "At that time, institutions serving predominantly minority students, especially the smaller institutions, had absolutely no ongoing research and had no research facilities. Now they are heavily involved in biomedical research, with the faculty publishing regularly in refereed journals and a significant number seeking regular research support from various institutes of the NIH and other sources besides the MBRS Program."

Research projects supported by the MBRS Program will be presented this month by 1,500 minority faculty and students at the annual MBRS symposium in Albuquerque, New Mexico. This dimension of symposium participation is in stark contrast to the limited involvement of minorities in biomedical research a decade ago. Then colleges with predominantly black, Hispanic, or other minority students did not have the capability to conduct biomedical research. This discouraged minority students from entering the field and placed those who did at a competitive disadvantage when they applied for advanced training. Also, faculty members at these institutions were frequently unable to engage in research because of heavy teaching loads and lack of necessary equipment and facilities.

To eliminate obstacles to minority participation in biomedical research, the MBRS Program was begun in 1972. It has provided research training to minority faculty and students and has provided MBRS institutions with needed facilities, equipment, and other resources to bolster their research capabilities. These currently well-equipped scientific settings provide the springboard from which increasing numbers of minority faculty and students are entering the mainstream of the nation's biomedical research effort.



MBRS students investigating the effects of polychlorinated biphenyls (PCB's), a group of poisonous environmental pollutants: ***research experience provides the foundation for admission to quality graduate schools.***

The number of eligible institutions receiving MBRS grants has increased from 38 in 1972 to 81 this year. The 81 funded institutions include 3 with a predominantly American Indian student body, 47 with a predominantly black student population, 4 with mostly Puerto Rican students, 2 with mostly Hawaiian students, and the remainder serving a mixed population of minorities in

PROGRESS *continued*

large metropolitan areas and in the southwestern United States. According to Dr. Gonzales, the research capability and productivity of these institutions has been bolstered not only by funding state-of-the-art research equipment and facilities, but also by hiring more research-oriented faculty capable of obtaining research grants and contracts and by holding workshops to improve faculty grantsmanship skills.

With the DRR Biotechnology Resources Program, the MBRS Program cofunded installation of the PROPHET system at five MBRS institutions. The PROPHET system is a highly integrated collection of powerful, computer-based tools for organizing, manipulating, analyzing, and communicating research data. The PROPHET system was used by 110 minority faculty and students this year to facilitate laboratory and clinical investigations. In another agreement, this one with the DRR General Clinical Research Centers Program, the MBRS Program cofunded a grant to one MBRS institution to develop its clinical research capability. It is hoped that this will eventually enable the grantee institution to compete successfully for General Clinical Research Center grants. The mainstreaming of MBRS institutions and minority faculty into regular NIH research has been accomplished through the MBRS Program's role as the cornerstone of the NIH extramural affirmative action program.

"In this capacity the program has established cooperative agreements with nearly all 11 institutes of the NIH and the National Institute of Mental Health to give these institutes a chance to tap this new pool of research talent," says Dr. Gonzales. The MBRS Program staff identifies MBRS research projects that appear to fit a particular institute's mission. If the institute approves the project and has funds available to support it, the institute transfers funds to the MBRS Program, which then acts as a clearinghouse and managing unit. By becoming acquainted with the institute's research focus and grant application requirements, MBRS institutions and investigators can eventually compete equally for institute grants through the individual research grant application procedure.

As part of its affirmative action efforts, the program also identifies qualified minority scientists for possible participation in NIH advisory and review committees. "Our program had a tremendous impact on the NIH by bringing to their attention quality minority investigators whom they didn't know about," comments Dr. Gonzales. "Prior to the MBRS Program, minorities were rarely involved in the NIH review process. Now there are approximately 40 minority scientists participating in NIH advisory and review committees. That is more than double the minority involvement of only a few years ago."

The overall research productivity and involvement of MBRS faculty investigators has markedly increased in the past 10 years due to MBRS funds, which provide them with release time from teaching to do research and enable them to attend scientific meetings and to be trained in needed

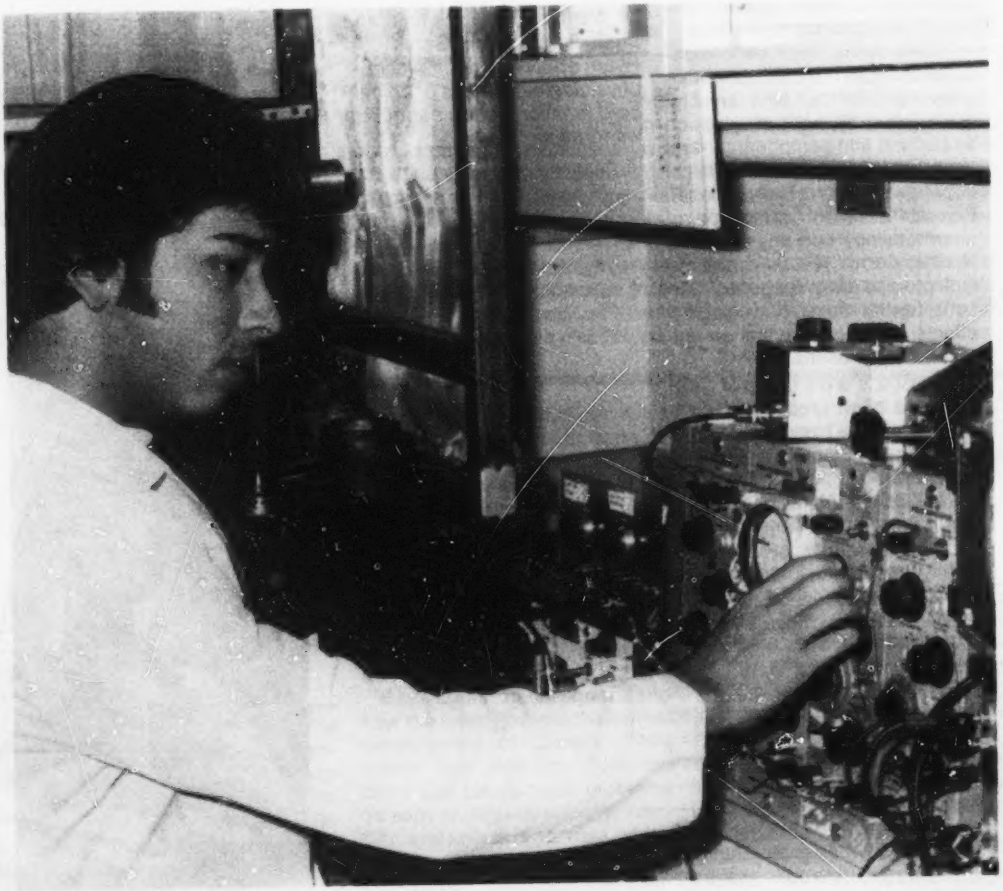
Dr. Ciriaco Gonzales, MBRS program director: ***"a quantum jump in the quality of biomedical research done by minority scientists."***

research skills. As a result, 636 minority faculty from MBRS institutions worked on 507 research projects in FY 1981. Data from FY 1980, the most recent available, indicate that MBRS faculty were involved in 318 reported projects, published 305 scientific papers, and made 757 presentations at scientific meetings. Several have been successful in obtaining research grants, and a significant number have obtained research support from the National Science Foundation and private organizations.

Research participation by minority students has greatly increased in the past decade as well. In fact, support of undergraduate research is a major focus of the MBRS Program. "By engaging minority undergraduates in biomedical research early, more become motivated to pursue biomedical research careers," asserts Dr. Gonzales. "Through our program, NIH funds were used for the first time to fund undergraduate research, and the benefits have been great. We're finding that more and more MBRS students are successfully gaining admission to graduate school or medical school because of their undergraduate research experience. Before the MBRS Program, it was rare for minority students to be accepted in the biomedical sciences at prestigious institutions. Now MBRS students are sought after because of their research experience."

Of the 4,500 minority students who have participated in the program since 1972, about 3,500 are pursuing or have pursued biomedical careers in graduate or medical schools. The most recent data from FY 1980 indicate that 499 MBRS students, including approximately 15 American Indians and 15 Hawaiians, received B.S. degrees. Of these, 129 entered medical school, 24 entered dental school, 137 went to graduate school, and 115 attended other health-related schools. In 1981, 80 percent of graduates from the four MBRS junior colleges transferred to 4-year schools to continue biomedical training. In the same year, 975 MBRS undergraduates and 368 MBRS graduate students participated in research activities and attended scientific meetings and symposia, and some coauthored scientific publications. Of the 368 graduate students, 10 received Ph.D. degrees.

"Now that we have more minority M.D.'s, we are hoping to provide them with incentives to pursue research instead of or in addition to more lucrative clinical practice," says Dr. Gonzales. "Another future objective is keeping up with the rest of the biomedical research community by providing appropriate animal facilities and more sophisticated equipment at MBRS institutions and by replacing wornout or obsolete equipment. The program will continue to provide opportunities to minorities wherever we can justify need for the program. This depends on what kind of populations an institution has and their special needs to become involved in research careers."



MBRS student examining neural electrical activity to identify underlying mechanism of epileptic convulsions: ***of the 4,500 minority students who have participated in the program since 1972, 3,500 have entered graduate school in biomedical science.***

Research Focus

Animal Model of Tranquilizer Dependence

An animal model for measuring dependence on benzodiazepines (BDZ's), a group of commonly prescribed tranquilizers, has been developed by a group of researchers at Northeastern University in Boston.

"Considering their very widespread usage, the BDZ's are considerably safer than other anxiolytic-sedative-hypnotic drugs, such as barbital and pentobarbital, and are less likely to produce physical dependence," says Dr. Norman R. Boisse, assistant professor of pharmacology and physiology at Northeastern. "We do, however, lack precise dose/response relationships for many physiological effects, and it is here that an animal model is useful."

The BDZ's are a group of related drugs often prescribed to reduce anxiety. They include the tranquilizers diazepam (Valium) and chlordiazepoxide (Librium), as well as the sleeping preparation nitrazepam (Mogadon).

Benzodiazepines are the most widely prescribed drugs in clinical medicine, according to the National Prescription Audit, 1980. More than 70 million prescriptions for these drugs were written in 1977 and an estimated 8,000 tons were consumed in the United States alone.

In studies with rats, Dr. Boisse found that profound tolerance—reduced sensitivity to the drug—developed over a period of 5 weeks in animals given chlordiazepoxide (CDP) in daily doses adjusted to produce a constant level of central nervous system (CNS) depression. This was evidenced by a fivefold increase in the total daily dose necessary to produce the effect. The increase in tolerance occurred most quickly over the first 3 weeks and then continued over the next 2 weeks without evidence of a ceiling or plateaulike phenomenon.

When the regimen was abruptly terminated all rats showed severe withdrawal symptoms. Signs seen included twitches, tremors, excessive muscle tension, arched back, high step, hair standing on end, apprehensive behavior, increased startle reaction, and weight loss. None exhibited seizures. The onset varied from 2 to 5 days. Peak withdrawal was maintained from the 6th day to the 11th day, and recovery was usually complete by 2 weeks after the las dose.

The symptoms observed were similar to those describing withdrawal from high doses of CDP in man. Dr. Boisse points out that although physical dependence on BDZ's has occurred with therapeutic dosages, dependence usually results from doses that greatly exceed those actually prescribed.

Dr. Norman R. Boisse (left) and predoctoral student Gary P. Ryan: ***observing rat in electrophysiological studies of spinal cord function during tranquilizer withdrawal.***

According to Dr. Boisse, this high-dose model of CDP tolerance and physical dependence provides an invaluable standard against which to compare other sedative-hypnotic drugs. He says the model is an essential tool for exploring and understanding the physiological mechanisms that underlie this tolerance-and-dependence state.

In their effort to develop a reliable model of BDZ tolerance and physical dependence in the Sprague-Dawley male rat, the researchers used the method of "maximally tolerable, chronically equivalent dosing." This procedure was originally developed by Dr. Boisse in collaboration with Dr. Michiko Okamoto of Cornell University Medical College to study barbiturate tolerance and dependence in the cat.

In this approach, the efficient induction of dependence is attempted by administering the drug in individually adjusted doses to ensure a continuous intoxication with a constant level of depression of CNS activity. CNS depression is measured on a numerical scale with respect to various gross neurological tests such as impairment of walking and suppression of motor activity and motor coordination in specific tasks.

In a comparative study with CDP and phenobarbital, Dr. Boisse found that tolerance to the barbiturate developed more slowly than for CDP. Equally effective doses had been increased only twofold at the end of the experiment as compared with a fivefold increase for the benzodiazepine.

The withdrawal reaction for phenobarbital, however, was generally more severe for most symptoms and included myoclonic jerks and seizures, which were absent with CDP. Phenobarbital withdrawal developed more rapidly with a 24-hour onset and 10 days to recovery. Dr. Boisse says that these observations support the predominant medical opinion that the long-acting BDZ's cause fewer and less severe withdrawal reactions than those associated with phenobarbital. Dr. Boisse believes that the results further suggest two fundamental differences between barbiturate and benzodiazepine dependencies. First, CDP tolerance develops faster than phenobarbital tolerance throughout long-term treatment. Secondly, the overt withdrawal reaction, while less severe for CDP, persists longer by as much as 4 days for one sign, hair standing on end, compared to phenobarbital.

These results, combined with the body of evidence supporting the hypothesis that BDZ's initiate their physiological response at specific binding sites, indicate that characteristic differences are likely, according to Dr. Boisse. He believes that cross-substitution and pharmacokinetic studies which are now in progress will help elucidate whether these differences are due to variances in drug response, drug elimination kinetics, or both.

Dr. Jerome Jaffe of the Columbia University College of Physicians and Surgeons has pointed out that withdrawal reactions for several drug classes are characterized by manifestations that suggest rebound hyperexcitation in those same physiological systems that were chronically depressed or altered by the drug. "It is here, in identifying relationships between withdrawal hyperexcitation and chronic depression," Dr. Boisse says, "that the chronically equivalent dosing model is well-suited for mechanistic study."

Numerous parameters of excitatory and inhibitory synaptic transmission have been explored by the researchers. Mr. Gary Ryan, a predoctoral student in Dr. Boisse's Laboratory, is currently conducting electrophysiological studies of the segmental reflexes of the spinal cord to determine how the balance between excitatory and inhibitory transmission may be changed and which properties of synapses are most prominently altered during BDZ withdrawal.

"The results indicate that both enhanced excitatory transmission and diminished inhibitory transmission contribute to the syndrome of nervous hyperexcitation," Dr. Boisse says. "It is interesting that the only synaptic parameters altered during withdrawal were those that were oppositely affected both acutely and chronically by the CDP regimen."

Accordingly, he believes these observations provide concrete evidence to support the hypothesis that rebound changes in synaptic transmission underlie the overt neurological signs and symptoms of withdrawal from long-term, high-dose benzodiazepine treatment.

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Research into the development of an animal model for benzodiazepine dependence was supported in part by the National Institute on Drug Abuse and the Biomedical Research Support Grant Program of the NIH Division of Research Resources.

Research into Neuroendocrinology: The Study of Diverse Hormonal Influences

This year marks the 20th anniversary of the General Clinical Research Centers (GCRC) Program, a program established to study and resolve significant health problems in the United States. This summary of research is based on a report submitted to the General Clinical Research Centers Program by the Advisory Group on GCRC Advances in the Neurosciences, chaired by Dr. Charles S. Hollander of New York University Medical Center.

Twenty years ago the pervasive influence of the human brain on the body's chemistry was attributed to hormonal reactions thought then to be independent of the central nervous system. Increasing knowledge of how these chemicals work has dispelled misconceptions, and research has documented the brain's control over diverse and seemingly unrelated actions in the body.

Physicians now understand how the brain governs the normal development of puberty. They realize that miscues from the brain can cause such problems as early or delayed puberty, infertility, and anorexia nervosa, which causes young women to starve themselves. Hormones that influence digestion and the occurrence of some nutritional diseases are known to be controlled by the brain. Even addiction to narcotics and the development of some types of diabetes now appear to have a neurological component.

These realizations grew from basic studies, many of which were carried out at the 73 General Clinical Research Centers spread across the United States. Through the GCRC Program of the NIH Division of Research Resources, these centers provide 75 percent of the research beds supported by the Public Health Service that are available outside the National Institutes of Health campus in Bethesda, Maryland, and are provided by the GCRC Program of the NIH Division of Research Resources.

It was in the GCRC's at the University of California in San Francisco (UCSF) and Montefiore Hospital in New York that researchers associated the prepupertal state with low-level secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). They noticed that nighttime bursts of gonadotropin from the anterior pituitary triggered gonadal secretion of sex steroids and produced the onset of puberty.

This provided the basis for work done in the GCRC's at the University of Iowa and the University of Michigan on anorexia nervosa, a disorder in which weight loss coincides with a reversion to the prepubertal pattern of gonadotropin secretion. The researchers demonstrated that proper nutrition in the anorexic child restores normal gonadotropin secretion and normal puberty.

In cases of puberty delayed by glandular malfunction rather than nutritional deficiency, small doses of the gonadotropin-releasing hormone LHRH (luteinizing hormone releasing hormone) may be used to prime the pituitary gland and begin the normal progression toward puberty. This therapy, developed just 9 years after LHRH was first isolated and characterized in 1971, has been successfully applied in the GCRC's of Massachusetts General Hospital and the University of Michigan.

Further study of the hormone led to the discovery that LHRH and its analogs desensitize the pituitary if given in large doses or by constant infusion. This phenomenon was used therapeutically by GCRC researchers at the Massachusetts General Hospital to shut down the abnormal early onset of puberty, a condition called idiopathic precocious puberty. The work was particularly significant in that no effective treatment for the disorder had previously been available.

Other disorders associated with the pituitary, particularly infertility, were scrutinized soon after prolactin, known as a hormone in lower animals for over 50 years, was clearly identified in man in 1970 as being separate from growth hormone. The development of sensitive bioassays and radioimmunoassays made it possible for several GCRC's to document the increase of prolactin in response to a variety of stimuli and events including stress, nursing in postpartum women, sleep, and the presence of dopamine antagonists.

This increase in secretion, the researchers learned, could be suppressed by dopamine, L-dopa, and other similar agents, a finding that proved useful for many women and men who were infertile due to overproduction of prolactin. European development of bromocriptine, a long-acting dopaminergic agent, led to a dramatic therapy for infertility. More than a thousand successful pregnancies have been reported following treatment with bromocriptine. A variety of GCRC's participated in the testing of this drug, which is now commercially available. Tests of other, longer-acting dopaminergic agents such as pergolide for the treatment of infertility are currently going on at GCRC's.

An unexpected finding that emerged from the prolactin studies at GCRC's was that a significant majority—two-thirds to three-quarters—of all pituitary tumors are associated with overproduction of prolactin. As a result, measurement of serum prolactin has become a useful diagnostic indicator of pituitary disease as well as a convenient way to follow the effects of therapy in patients with elevated levels of the hormone.

A very recent finding, reported by researchers in Europe and by American researchers in GCRC's including the one at New York University Medical Center, has been that bromocriptine not only lowers prolactin secretion in the case of pituitary tumors but also shrinks some of these tumors. Although the effects appear to last only as long as the drug is administered and long-term hepatic toxicity may prove to be a limiting factor, the observation raises the possibility of nonsurgical treatment.

A basic concept, suspected since 1935 and firmly established some 10 years ago, describes the interaction of the pituitary with the hypothalamus and the thyroid gland. The regulation of the hypothalamus by the anterior pituitary was recognized initially by observing patients with hypothalamic lesions. Ultimately researchers isolated and identified various associated hormones including somatostatin (somatotropin-releasing inhibitory factor).

Somatostatin was first isolated in 1973. Animal tests showed that it not only inhibits the production of growth hormone but lowers blood sugar, an event later traced to its inhibition of glucagon. Soon researchers learned that somatostatin also inhibits the secretion of insulin.

Tests done in the GCRC at the University of California, San Francisco, showed that the infusion of somatostatin in amounts that have no significant side effects reduced the blood level of glucagon in juvenile diabetics who require insulin and that this leads to lower blood sugar. Having demonstrated the role of glucagon in glucose homeostasis, the GCRC researchers demonstrated that the occurrence of hyperglycemia and ketoacidosis in insulin-requiring diabetics following insulin withdrawal could be markedly reduced if glucagon infusions are given simultaneously with somatostatin. These investigators next showed that administering somatostatin with insulin reduced post-treatment variability of blood glucose in juvenile diabetics.

But researchers at the Yale University GCRC soon showed that the disease in diabetics who have a moderate amount of residual insulin secretion is aggravated by administering somatostatin. They also demonstrated that somatostatin inhibits the absorption of glucose and amino acids from the gut. These are important findings not only in that they describe the physiology of somatostatin, but because they are a necessary prelude to the introduction of somatostatin into clinical practice.

Unlike the naturally occurring hormone, the newly recognized analog somatostatin-28 has been shown to have a greater suppressive effect on insulin than on glucagon, indicating that this analog may be more clinically useful. Studies of somatostatin analogs that can be taken by mouth and of longer lasting analogs created by peptide engineering are also in progress at GCRC's.

Although somatostatin in its natural form will probably not have much clinical value, the work done with this material indicates that somatostatin secretion plays an important role in normal human physiology and that the chemically modified hormone may have clinical benefit.

Research into the hypothalamic-pituitary-thyroid axis expanded enormously with the isolation, characterization, and synthesis in 1971 of the thyroid hormones thyroxine and triiodothyronine. Research on thyrotropin-releasing hormone (TRH) became possible with the advent of radioimmunoassays for thyrotropin (TSH).

Thyroxine, commonly abbreviated as T_4 since it contains four iodide atoms, constitutes the major hormonal secretory product of the normal human thyroid gland. Once secreted, T_4 affects virtually every organ and tissue in the body. The secretion of this hormone by the thyroid gland is normally regulated by the level of pituitary TSH in the blood. Triiodothyronine, or T_3, which contains three iodine atoms, is the more active thyroid hormone. T_3 and T_4, while circulating in blood, are usually bound to proteins of which thyroxine-binding globulin appears to have the leading role.

In the late 1960's researchers at the University of Rochester developed a gas chromatographic method for measuring T_3. Using the GCRC at Rochester and later the one at New York University Medical Center, they defined a form of T_3 toxicosis—hyperthyroidism caused by an excess of T_3 secretion. These pioneering studies demonstrated the important role of T_3 in thyroid hormone metabolism and its major contribution to the metabolic activity of the thyroid secretions. Further understanding of the role of T_3 was aided by the development of radioimmunoassays for

T_3 by these same GCRC researchers and other investigators. With these assays, it was demonstrated that much of the circulating peripheral T_3 is converted from T_4 to T_3. This conversion produces a four- to tenfold increase in hormonal activity.

A variety of conditions have been described that can best be grouped under the general heading of "low T_3 syndromes." These include aging, caloric deprivation, and many acute and chronic nonthyroidal illnesses in which there appears to be abnormally low conversion of T_4 to T_3.

Of considerable clinical value is a spinoff from the development of the T_3 radioimmunoassay, namely, the development of T_4 radioimmunoassays, which are gradually replacing T_4 measurements made by competitive binding analysis in many clinical laboratories throughout the country. This newer methodology has allowed practical screening techniques for the detection of neonatal hypothyroidism.

Many of the metabolic actions of thyrotropin (TSH) have been studied with a biologic assay which, through its use in GCRC's, has been recognized as a useful means of diagnosing even mild degrees of primary hypothyroidism. It can be used in the early detection of thyroid failure and as an objective measure for determining the adequacy of thyroid hormone replacement therapy. Additionally, its use in neonatal screening for hypothyroidism complemented the serum T_4 radioimmunoassay measurement in pinpointing those patients most likely to have inadequate thyroid gland function.

An enormous public health benefit of the assays for T_3, T_4, and TSH and their refinements for microassays on dried blood spots has been the development of widespread screening of newborns for primary hypothyroidism. One in 5,000 newborn infants in North America has hypothyroidism which, if not properly treated, will result in permanent mental retardation, according to researchers. These observations indicate that neonatal hypothyroidism may be one of the most common treatable causes of mental retardation, having an incidence several times greater than phenylketonuria or galactosemia. Researchers actively using GCRC's have been prominent in the development of these assays.

Although the gastrointestinal (GI) tract is not usually thought of as an organ responsive to hormones, research indicates that it is Over the last 15 years investigators have found that many, if not all, of the peptides in the gut are also present in the brain, although the precise functions of most of them in the brain are still unknown. The potential number of hormones affecting a variety of functions of the GI tract far exceeds the large number of hormones identified as originating from the classic endocrine glands.

The confirmed and suspected hormones of the GI tract are anatomically distributed much differently than hormones of classic endocrine glands such as the pituitary or parathyroids. Cells containing GI hormones are widely and sparsely distributed in cells lining the stomach, small and large bowel, and pancreas. Also, polypeptides secreted by some of the cells directly affect the function of adjacent cells, whereas hormones of the endocrine glands are transported to their target tissues by the bloodstream.

Gastrin, perhaps the most thoroughly evaluated GI hormone, is synthesized and secreted by G cells found in the distal part of the stomach called the antrum and the first part of the duodenum. Gastrin, released from the lower part of the stomach, stimulates parietal cells located in the upper part of the stomach, enhancing their secretion of acid. Increased acid secretion, in turn, inhibits or decreases gastrin secretion by cells at the lower part of the stomach. Gastrin also stimulates pepsin secretion and through some unknown mechanism affects growth of the cells that line the stomach.

GCRC studies into the control of gastric acid secretion have already been applied toward the development of specific therapeutic agents such as blockers of histamine receptors. These blocking agents have proved useful in the treatment of various disorders of gastric acid hypersecretion and related peptic disease. Some of these investigations also related the brain and central nervous system to the role of peripheral peptide hormones in both normal physiology and pathologic states.

Researchers again ventured outside the confines of classic neuroendocrinology when they pursued the hypothesis that metabolic imbalance was at least partly responsible for addiction to narcotics. This led investigators at the Rockefeller University Hospital GCRC to develop a pharmacologic maintenance treatment for heroin addiction.

They started with the assumption that an alternate opioid might avoid withdrawal symptoms in a street addict and make it possible to rehabilitate him. At the same time the investigators recognized that, to avoid the use of needles, such maintenance treatment would need to be performed with an orally effective drug. More important, treatment would entail use of a long-acting narcotic to allow a steady state to be established with respect to drug disposition and restoration of normal physiological and behavioral functions. The orally effective narcotic methadone, which acts for 24 to 36 hours, fits these criteria.

As a result of the studies performed in the Rockefeller GCRC, it was shown that this drug, when given orally once a day, could prevent all symptoms of narcotic withdrawal. Methadone also prevented "drug hunger," a phenomenon now recognized as the leading cause for rehabilitated addicts to return to heroin. The studies showed that the dosage of methadone did not have to be increased to provide these desired effects. Once tolerance to all acute and subacute narcotic effects was achieved, the patients in treatment were essentially normal, as judged by objective criteria. In a sequence of double-blind studies it was also shown that during daily high-dose methadone treatment, a sufficient degree of tolerance and cross-tolerance to heroin is achieved so that patients receiving treatment could not recognize the administration of even large doses of heroin or morphine and that no objective changes occurred during intravenous administration of narcotics.

Subsequent studies were carried out by this same group of researchers to develop procedures for chronic treatment of addiction with methadone and for determining the safety and efficacy of such treatment. Also, medical safety and side effects during methadone treatment were assessed along with methods for reversing narcotic overdose.

Methadone maintenance treatment has now been given to more than 120,000 patients in the United States. About 70 percent of the patients receiving this treatment have continued in the program and stopped using illicit narcotics.

However, caution is necessary when administering methadone because the drug is addicting, and it carries the potential for abuse. These risks occur primarily when methadone is diverted to the streets where addicts use it without medical supervision.

Methadone research and studies into physiologic and pathologic states that naturally occur could not have been done without being aware that neurotransmitters and neuropeptides have effects that reach far beyond neural tissue. The results from these studies have provided an understanding of human disease in many areas outside the discipline of neuroendocrinology and promise a continuing growth of knowledge in the coming years. **R**

The in-depth report on research into neuroendocrinology and how this discipline relates to diseases not classically associated with the brain is available from the GCRC Program of the NIH Division of Research Resources.

For a copy of the report, write to:
Dr. William DeCesare
Director, GCRC Program
Room 5B-51
Building 31
National Institutes of Health
Bethesda, Maryland 20205

Research Resources Reporter
April 1982

Information Exchange

Research Highlights

At the Johns Hopkins University in Baltimore, Maryland, Dr. Michael Beer and his associates are using heavy atoms to label biological molecules for examination with a high-resolution scanning transmission electron microscope (STEM).

According to Dr. Beer, professor of biophysics and principal investigator at the Atomic Microscopy of Labeled Systems: A STEM Facility, labeling molecules with heavy atoms such as platinum or osmium may allow scientists to study biological structure and to understand the mechanisms that control the activities of biological systems.

"We attach heavy atoms to molecular constituents to mark structures of interest. By locating these markers, we can deduce the position of the labeled molecules," says Dr. Beer.

With the STEM, the researchers have learned about the architecture of nucleosomes, protein structures that contain the genetic material of eukaryotic cells. These cells make up the body tissue of plants and animals. The STEM is also being used to study collagens, which are the structural proteins of connective tissues, and to study muscle filament. Nucleic acids and proteins such as these have been extensively examined with low-resolution electron microscopy and X-ray diffraction. "These techniques have revealed a wealth of structural information of cell properties, but there are many biological problems for which such methods are inadequate, especially studies of materials that are not easily crystallized," says Dr. Beer. "For these structures, a more detailed analysis can be obtained with the STEM."

In STEM, a finely focused electron beam moves from one point of the specimen to the next to form a pattern similar to television imaging. Information about the specimen is obtained through a detailed analysis of the perturbations suffered by the beam electrons in their collisions with the atoms and molecules of the specimen. The energy lost by the electrons indicates their excitation energies. The electron bombardment causes different parts of the cell to absorb or scatter electrons in different ways to form an image on an electron-sensitive plate. The electron beam in a STEM allows several hundred times more electrons to be focused into a small spot than is possible with the source of a conventional scanning electron microscope. The Johns Hopkins STEM can resolve details down to 2.5 angstroms.

Using the STEM and heavy atoms to label nucleosome constituents, Dr. Beer and his associates have determined the positions of histones, the protein components of nucleosomes. "Our findings may help biophysicists better understand the process of gene control," says Dr. Beer. Research by other investigators indicates that inactive chromosomes consist of coiled strings of nucleosome granules. When genes are activated, these structures ap-

pear to uncoil and the nucleosomes may rearrange into a more open configuration.

Scientists know that nucleosomes are cylindrically shaped complexes of eight histones, which look like beads on a string, with each bead being a nucleosome covered and connected by DNA. But until now the arrangement of the histones has been unclear.

The researchers separated the histones after attaching them to labels of platinum atoms coupled with the peptide dye glycyl-L-methionine. They then reassembled the nucleosomes with some of the histone pairs. By examining the reconstructed particles in the STEM, the researchers could observe the labels and infer the location of the histone pairs.

In other STEM research, the investigators have successfully labeled monoclonal antibodies with heavy atoms. In collaboration with Dr. Tom Pollard of the Johns Hopkins University, they will use these complexes to learn more about the structure of myosin filaments. In preliminary tests, the researchers have shown that labeled antibodies can be easily seen at high resolution through the STEM. "This method is superior to conventional techniques in which scientists indirectly label antibodies with huge molecules called ferritin, which ultimately leads to a loss of resolution," says Dr. Beer. "Labeling antibodies with heavy platinum atoms for the STEM minimizes this loss."

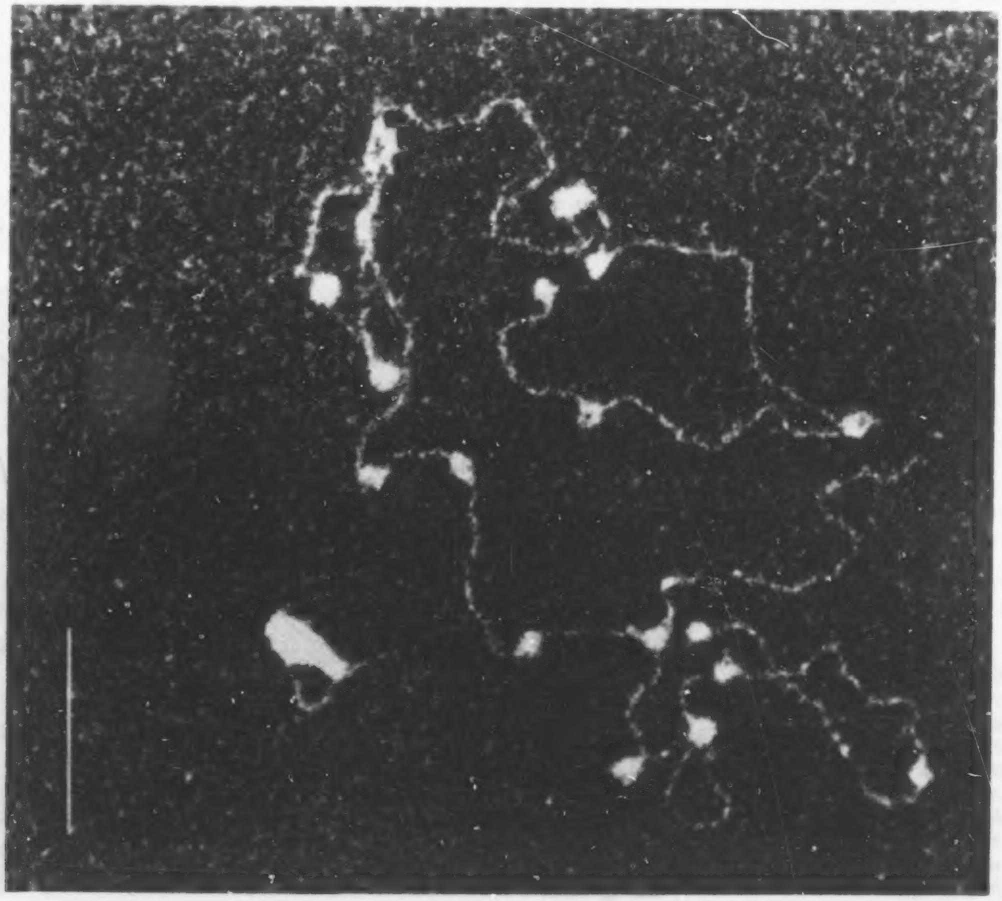
Heavy atom labeling procedures are also being used to analyze features of collagens, the abundant proteins of connective tissue. At least four types of collagen exist, each with a characteristic amino acid sequence. However, the function of each type of collagen is unclear. Using a platinum dye, which is specific for the sulfur-containing amino acids, and an osmate dye, which is specific for the sugar residues attached at specific sites, the researchers are staining individual collagen molecules extracted from calf and rat tendon and skin and cartilage tissue, to see if the positions of amino acids in proteins can be inferred from the observed labels. Preliminary observations with the STEM suggest that there are recognizable differences in the labeled collagens, which might be used for identification, says Dr. Beer.

Construction of the Johns Hopkins University's STEM began in 1971. Resolution below three angstroms, which is necessary for heavy atom detection, was achieved in 1976. Practical use of the STEM did not begin until late that year. Since then, the resource has been used frequently by investigators from the Johns Hopkins University and by visiting investigators. A liquid helium-cooled stage and a high-performance electron energy-loss spectrometer, which will provide increased chemical information, are currently being assembled.

Scientists are encouraged to visit the facility, and the hours and days that the instrument is available for research are flexible. Off-campus housing and meals are available at moderate cost. Projects are selected from proposals reviewed by the staff and the resource's advisory committee.

"STEM operators are available to visiting scientists, so it is not necessary to be specifically trained in STEM techniques," Dr.

Dr. Chris Stoeckert (left) and Dr. Michael Beer: ***analyzing biological molecules with the scanning transmission electron microscope (STEM).***



STEM micrograph of nucleosomes connected by DNA: ***revealing structural information on specimens that are not easily crystallized and are, therefore, difficult to study by methods other than STEM.***

Beer says. "We also provide many of the things required for specimen preparation, such as embedding, sectioning, and evaporating equipment."

The Johns Hopkins University STEM is one of the world's only instruments that enable the study of single atoms and biological molecules tagged with heavy atoms. A STEM with liquid nitrogen cold stage and specimen changer is also available at the Brookhaven National Laboratory in Upton, New York. The first STEM for biological microscopy, which was developed by Dr. Albert V. Crewe and his colleagues at the University of Chicago Enrico Fermi Institute, served as the model for the resource at the Johns Hopkins University.

Additional reading:

1. Beer, M., Wiggins, J., Stoeckert, C., Marenus, K., Kuhn, E., and Erickson, M., STEM studies of biological structure. *Ultramicroscopy*. In press.
2. Beer, M., Carpenter, R., Eyring, L., Lyman, C., and Thomas, J., Chemistry viewed through the eyes of high-resolution microscopy. *Chemical and Engineering News* 59:40-61, 1981.
3. Beer, M., Wiggins, J., Tunkel, D., and Stoeckert, C., Biological structure determination through atomic microscopy. *Chemica Scripta* 14:263-266, 1978-79.

Dr. Beer's use of STEM to study biological structure and function is supported by the National Science Foundation and the NIH National Institute of General Medical Sciences. The Atomic Microscopy of Labeled Systems: A STEM Facility is supported by the Biotechnology Resources Program (BRP) of the NIH Division of Research Resources. The Brookhaven National Laboratory is also funded by the BRP. The STEM developed by Dr. Crewe was originally supported by the BRP. His research now is supported by the Department of Energy.

Research Service

Miniature Electronic Sensors

Engineers at the Resource for Silicon Biomedical Sensors at Stanford University are developing and fabricating miniature electronic sensors to measure a wide range of biological parameters including pressure, force, motion, and temperature distribution. These sensors, which are integrated circuitry, are designed to meet the specifications determined by the needs of individual biomedical research projects being done by investigators both within and outside Stanford University.

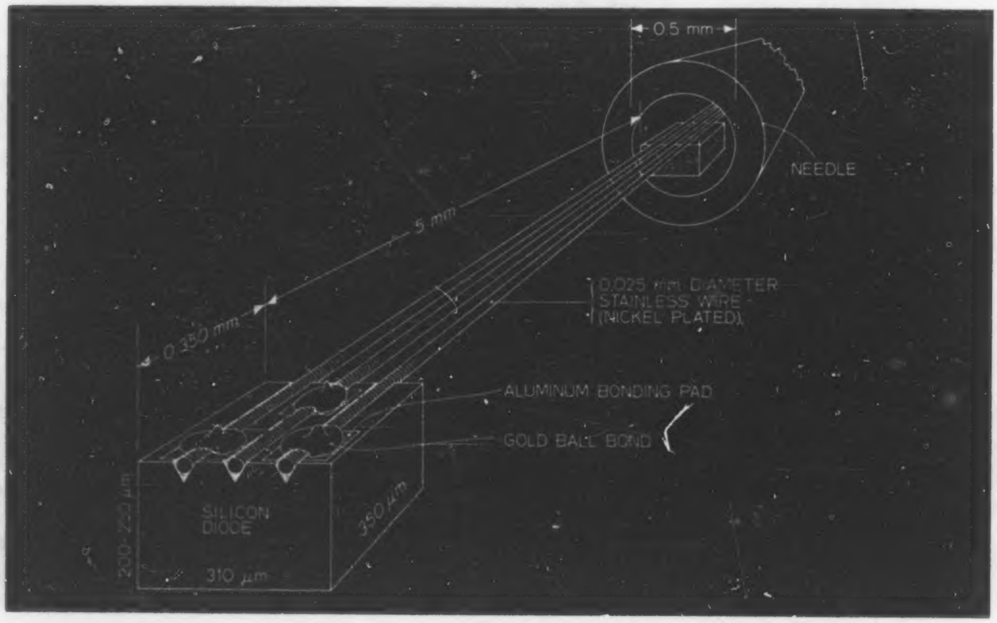
Because the sensors are very small, lightweight, and require little electrical power, they can often be implanted at the exact location needed to measure the desired parameters. Their size and design cause very little tissue distortion or local heating, thereby causing little or no measurement error due to their presence.

In response to a request by medical researchers and physicians who are treating cancer with hyperthermia (elevated temperature induced by ultrasound or ultra-high-frequency radio waves), an array of temperature sensors is being developed to measure temperature distribution within tissue during treatment. Hyperthermia of malignant tissue shows great promise for selectively killing cancer cells, either alone or in combination with radiation or chemotherapy. By implanting the sensor array in the tumor, the physicians can accurately monitor the heat distribution throughout the tumor and surrounding regions during treatment. Both the effectiveness and control of hyperthermia are greatly enhanced by knowing this temperature distribution.

Earlier, in a sensor project not supported by the resource, the same team of engineers designed an array of pressure sensors and associated signal processing electronics that was packaged and worn like a wristwatch to constantly record blood pressure readings for later analysis. It was developed primarily for the study of various factors affecting high blood pressure.

Core research at the resource is focused on finding new and better means of packaging and fabricating sensors for greater accuracy and long-term stability. In particular, the use of integrated circuitry for signal-processing placed directly on the sensor element is being investigated as a means of achieving a higher level of stability than is currently possible. The circuitry encodes the sensor output in such a way (for example, digital "words") that changes in resistance, leakage, or position of the leads to the sensor will have no effect on the output reading. In addition, such circuitry will often make it possible to reduce the number of electrical connections to a sensor, according to Dr. James Angell, principal investigator of the resource.

The resource was established in September 1977 in the integrated circuit (IC) laboratory of the Stanford electronics laboratories,



Diode temperature sensor developed at the Resource for Silicon Biomedical Sensors: ***when implanted in a cancer tumor, the sensor provides accurate measurements of heat distribution in the tumor and surrounding tissue during treatment with ultrasound or ultra-high-frequency radio waves.***

which comprise the research component of the electrical engineering department in the Stanford School of Engineering. Dr. Angell reports to Dr. James D. Meindl who directs the IC laboratory and the Stanford electronics laboratories.

Many pieces of equipment at the resource are shared with researchers elsewhere in the IC laboratory and, conversely, many facilities of the IC laboratory are used by resource personnel in the design, fabrication, and testing of sensors. As such, the resource is part of the IC laboratory, but its programs and goals are independently determined, except as prescribed by cooperative agreements concerning expertise and equipment.

Each new sensor built at the resource is developed through close cooperation between the collaborator and technical staff members. Engineers and technicians at the resource are available to help collaborating scientists understand the characteristics of materials, the operation of electronic measuring equipment, and the techniques for constructing new sensors. Bench space is provided to users of the laboratory.

Those wishing to learn how to apply sensors that have already been developed can be trained by resource staff members and biomedical collaborators in handling, connecting, and measuring with the sensors. Reports and recommendation sheets, which accompany sensors, are well documented and easy to understand. Library reports and instructions concerning the sensors are also available. Periodic seminars deal with technical aspects and applications of sensors. There are no service fees for collaborators and users.

For further information, contact:
Dr. Phillip W. Barth
Room 124
Applied Electronics Laboratory
Stanford University
Stanford, CA 94305
Telephone (415) 497-2596

Meetings

Course in Polymeric Delivery Systems to Be Held

"Controlled-Release Technology: Polymeric Delivery Systems for Drugs, Pesticides, and Foods" is the title of a summer course to be held at the Massachusetts Institute of Technology (MIT) from July 19 to 23, 1982. The course will cover recent advances in controlled-release technology and will explain polymer systems as they relate to achieving appropriate design of controlled-release and liposomal functions. The course will be presented by faculty from MIT and other universities who are currently active in the topics to be covered. For further information write to:

Director of Summer
Session Office
Room E19-356
Massachusetts Institute
of Technology
Cambridge, Massachusetts
02139

Conference on Immunofluorescence and Immunoenzymes

The 8th International Conference on Defined Immunofluorescence, Immunoenzyme Studies and Related Labeling Techniques will be held in Niagara Falls, New York, from June 8 to 11, 1982. Plenary sessions, symposia, and workshops will review state-of-the-art applications of immunofluorescence, immunoenzyme studies, and techniques related to specific fields of study. Workshops will address application of these techniques to autoimmunity, biophysics/biochemistry, dermatology, endocrinology, microbiology, nephrology, neurology, oral biology, physiology, and virology. For additional information, contact:

Dr. Ernst H. Beutner
State University of New York
at Buffalo
School of Medicine
219 Sherman Hall
Buffalo, New York 14214
Telephone (716) 831-2905

Symposium on Extracellular Matrix

The 14th Michigan Molecular Institute Symposium will be held from June 28 to July 2, 1982, in Midland, Michigan. The topic is the extracellular matrix (ECM), with the following issues to be discussed: structure and functional interactions of the ECM components; systems for studying the role of ECM in growth and differentiation; diseased states of ECM; and methods for culturing tissues and cells on surfaces derivatized with ECM and other defined components. For additional information, contact:

Symposium Secretary
Michigan Molecular Institute
1910 West St. Andrews Road
Midland, Michigan 48640
Telephone (517) 631-9450

April 16-17, 1982

Title: *4th Annual Neonatal/Perinatal Spring Symposium: Infectious Diseases*
Location: *Ontario, Canada*
Contact: *Rayna Dutton*
Pediatric Continuing Medical Education
Children's Hospital
219 Bryant Street
Buffalo, New York 14222
Telephone (716) 878-7630

April 18-24, 1982

Title: *Symposium on Highlights in Modern Immunology*
Location: *Budapest, Hungary*
Contact: *Professor G. Gy Petranyi*
c/o MOTESZ Congress
Bureau Budapest
P.O. Box 32
1361 Budapest, Hungary

April 22-24, 1982

Title: *International Congress on Access Surgery*
Location: *Maastricht, The Netherlands*
Contact: *ICAS Secretariat*
Department of Surgery
State University of Limburg
St. Annandel Hospital
Annandel 1, 6214PA Maastricht
The Netherlands

April 23, 1982

Title: *Sensory-Evoked Potentials in Pediatrics*
Location: *Washington, D.C.*
Contact: *Susan Weiss*
Convention Services
Children's Hospital National Medical Center
111 Michigan Avenue, N.W.
Washington, D.C. 20010
Telephone (202) 745-3000

April 25-29, 1982

Title: *31st Annual Scientific Session of the American College of Cardiology*
Location: *Atlanta, Georgia*
Contact: *Lawrence H. Leong*
American College of Cardiology,
9111 Old Georgetown Road
Bethesda, Maryland 20014
Telephone (301) 897-5400

April 29-May 1, 1982

Title: *Symposium of Clinical Vision Research: Epidemiologic and Biostatistical Approaches*
Location: *Sarasota, Florida*
Contact: *Catherine M. Beinhauer*
Epistat Associates
Norwich, Vermont 05055
Telephone (802) 295-6377

May 11-14, 1982

Title: *Annual Meeting of the Society for Pediatric Research*
Location: *Washington, D.C.*
Contact: *J. D. Johnson, M.D.*
Department of Pediatrics
University of New Mexico
School of Medicine
Albuquerque, New Mexico
87131
Telephone (505) 277-4361

May 11-14, 1982

Title: *Transport ATPases*
Location: *New York, New York*
Contact: *Conference Department*
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021
Telephone (212) 838-0230

May 13, 1982

Title: *Exploring Research Approaches to Psychotherapy*
Location: *Bethesda, Maryland*
Contact: *Dr. Lyle W. Bivens*
Deputy Director
Division of Extramural Research Programs
National Institute of Mental Health
Rockville, Maryland 20857
Telephone (301) 443-3563

May 16-20, 1982

Title: *5th International Symposium on the Prevention and Detection of Cancer*
Location: *Sao Paulo, Brazil*
Contact: *Medical Congress Coordinators Department*
1212 Avenue of the Americas
New York, New York 10036
Telephone (212) 840-0110

May 18-21, 1982

Title: *5th International Conference on Prostaglandins*
Location: *Florence, Italy*
Contact: *Fondazione Giovanni Lorenzini*
Via Monte Napoleone
23-20121 Milan, Italy

May 19-22, 1982

Title: *International Symposium on Leukemia Cell Biology and Therapy*
Location: *Memphis, Tennessee*
Contact: *S. B. Murphy, M.D.*
St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, Tennessee 38101
Telephone (901) 552-0300

May 24-28, 1982

Title: *Reconstructive Microsurgery Symposium and Workshop*
Location: *Oklahoma City, Oklahoma*
Contact: *H. Vorse, M.D.*
Continuing Medical Education
13th and Lincoln Boulevard
Oklahoma City, Oklahoma 73104
Telephone (405) 271-6447

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Research Resources Reporter

May 1982, Volume VI, No. 5

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and Biomedical Research Support

This Issue:

Male Sex Determination
Lithium Studies
Relaxation Response
Powerful NMR Spectrometer

by
Greg Freiherr

Biochemical Changes In Developing Cancer Cells

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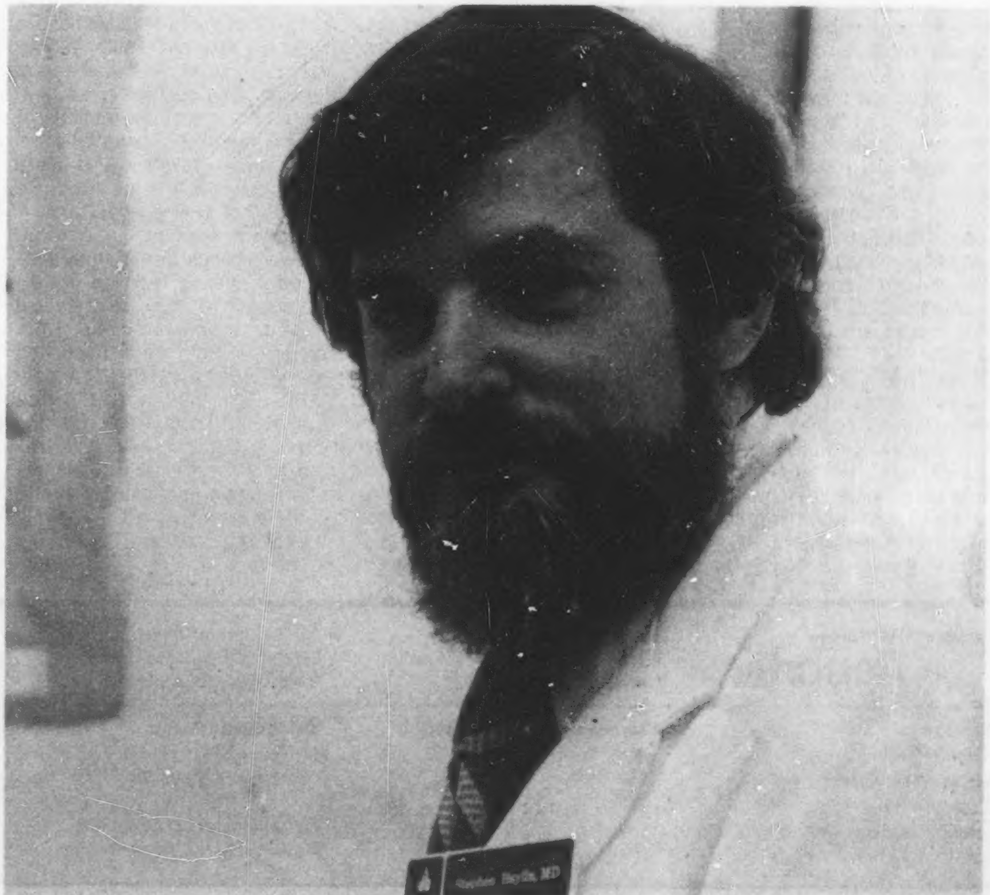
Some cancer cells change biochemically as they develop, and these changes correlate with increasing virulence of the cancer and worsening of the prognosis, according to researchers at Johns Hopkins University.

Their conclusions, based on a recent study done at the Johns Hopkins General Clinical Research Center, agree with the widely discussed hypothesis of tumor progression. That hypothesis describes cancer cells as being biochemically homogeneous or similar in early stages of the disease and becoming heterogeneous over time. This progression has been well documented in animal models but not in human cancers, which are usually discovered in an active stage, after cells are already biochemically mixed.

However, the Hopkins team, led by Drs. Stephen B. Baylin and Geoffrey Mendelsohn, has described this progression in a subset of patients who are severely affected by inherited medullary thyroid cancer (MTC), a cancer that can be tracked from its



Dr. Stephen Baylin of Johns Hopkins University: ***"We are not talking about a cure but, rather, survival."***

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CANCER CELLS *continued*

earliest stages. The disease is well suited for exploring the behavior of human cancer cells over time, because it is often detected early through family screening.

"The exciting question to ask is whether this cancer reflects the progression of events that contribute to cellular heterogeneity in all cancers," says Dr. Baylin, associate professor of oncology and medicine. "I think the answer is that cell mixtures of the type we have found in some patients with MTC are present in most types of cancer and that the progression of MTC is typical."

Most cancers now being carefully studied, including cancers of the lung and prostate, show heterogeneous tumor cell populations. "And if these tumors could be examined at an early stage, when the tumors are microscopic, we suspect that they would be more homogeneous than in later stages," he says.

The Hopkins study indicates that medullary thyroid tumors containing a mixture of cells with extreme biochemical differences are much more likely to spread widely and aggressively than homogeneous tumors. Dr. Baylin and his colleagues have not specifically identified why cell variation leads to increased virulence or why it begins. They have, however, drawn some general conclusions.

"We have indications that there's some sort of maturation block, that the more aggressive cells—those with the greatest biochemical differences—remain in an immature form, failing to express the biochemistry of the mature parent cells," says Dr. Baylin, whose specialty is tumor endocrinology. "If we can understand why aggressively growing cancer cells express this different pattern of biochemistry, we may learn why they grow so rapidly."

The Hopkins researchers have been interested primarily in hereditary MTC because routine family screening identifies patients in early stages of the disease. Early tumors can then be compared to tumors in later stages. The hereditary form accounts for 20 percent of all MTC cases, or about 200 cases per year in the United States, according to Dr. Baylin. Many victims are children. The only effective treatment at present is surgical removal of the thyroid.

Although patients with the nonhereditary form of the disease will benefit from the knowledge gained by Dr. Baylin and his colleagues, those patients with inherited MTC stand to gain the most because their cancer is often discovered early. Dr. Mendelsohn says: "Probably the most immediate clinical impact will be that patients and physicians may feel more comfortable with conservative surgical management of patients when the tumors removed are homogeneous. The question of what to do if the cancer is heterogeneous has not been answered."

Although MTC tumors in early stages of the disease often spread locally, most of these patients do well. They appear to be the ones with biochemically homogeneous tumors. Those with heterogeneous tumors tend to develop widespread metastases, which can cause death. Chemotherapy and radiation have proven ineffective for these patients, says Dr. Mendelsohn. If, however, the physician knew early that the cancer had a strong potential for wide dissemination throughout the body, greater effort might be applied to looking for metastases and considering aggressive treatment, says Dr. Baylin.

As support for employing a flexible treatment regimen, Dr. Baylin cites the high mortality found in the Hopkins study among patients with biochemically mixed tumor cells, compared to patients with homogeneous tumors. Of six patients who had mixed cancer cells, five died within 5 years after treatment began. Of the 11 participants with homogeneous cells, all have survived for periods up to 17 years.

"This does not imply that surgery cures the disease in patients with homogeneous tumors," says Dr. Baylin. "It only shows that these patients have a strikingly different clinical course than patients with heterogeneous tumors. The patient who has lived 17 years since removal of the primary tumor has considerable tumor growth in the chest and neck, but it's localized. So we are not talking about a cure but, rather, survival."

Two siblings in the study provided a striking example of how variations in the biochemical makeup of tumors correlate with prognosis. Both were diagnosed as having the inherited form of MTC. Both had similar symptoms, and both received the same treatment—surgical removal of the thyroid. Six months after surgery, the younger sibling has no trace of MTC, whereas the other's cancer has spread to the liver.

The most significant difference between the two siblings, aside from a 5-year difference in age, was the composition of their tumors. The younger patient had tumor cells that were biochemically similar; the sibling whose cancer spread to the liver had biochemically mixed cells.

All patients participating in the study were identified through routine family screening with a special blood test for serum calcitonin. Soon after physicians detected abnormally low levels of this hormone, which is produced by the thyroid, they removed the gland. By studying the biochemistry of tumors removed from patients at different stages of the disease, the researchers were able to document a progression of biochemical changes.

To determine tumor cell composition, the research team mapped the calcitonin concentration in individual MTC tumor cells. They found that the concentration of the hormone was homogeneous from cell to cell in normal tissue from which the tumor arose and in tissue with only traces of the disease. In some patients with more advanced, proliferating cancer, the cells—though they appeared similar—showed extreme variations in calcitonin concentration. Many cells in the most virulent MTC tumors showed little or no trace of the hormone.

Not only does the concentration of calcitonin decline as cells become more virulent, but its ratios to the concentrations of two other tumor markers—L-dopa decarboxylase and histaminase—become extremely low. Because very high calcitonin concentrations occur as normal

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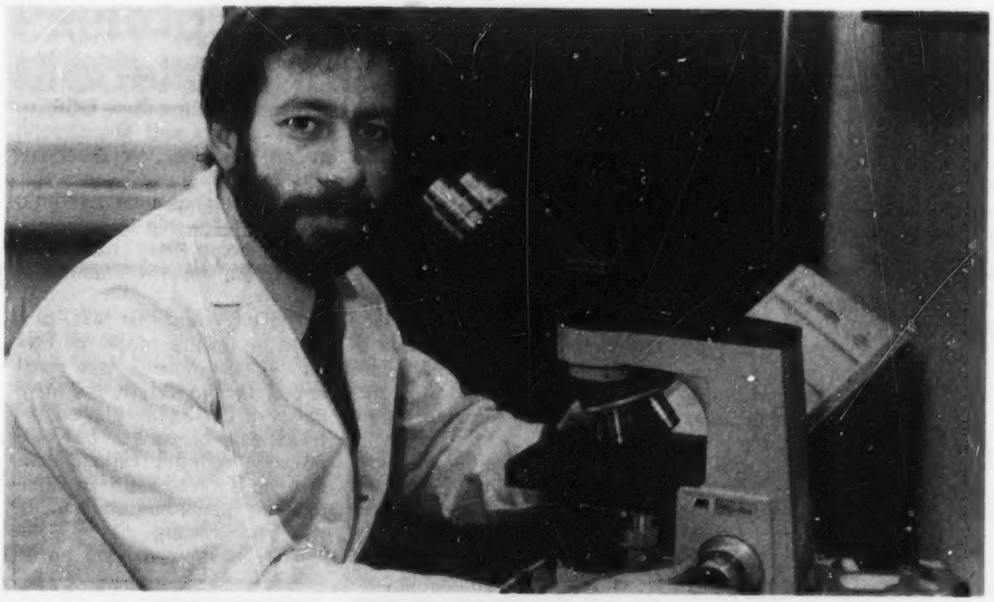
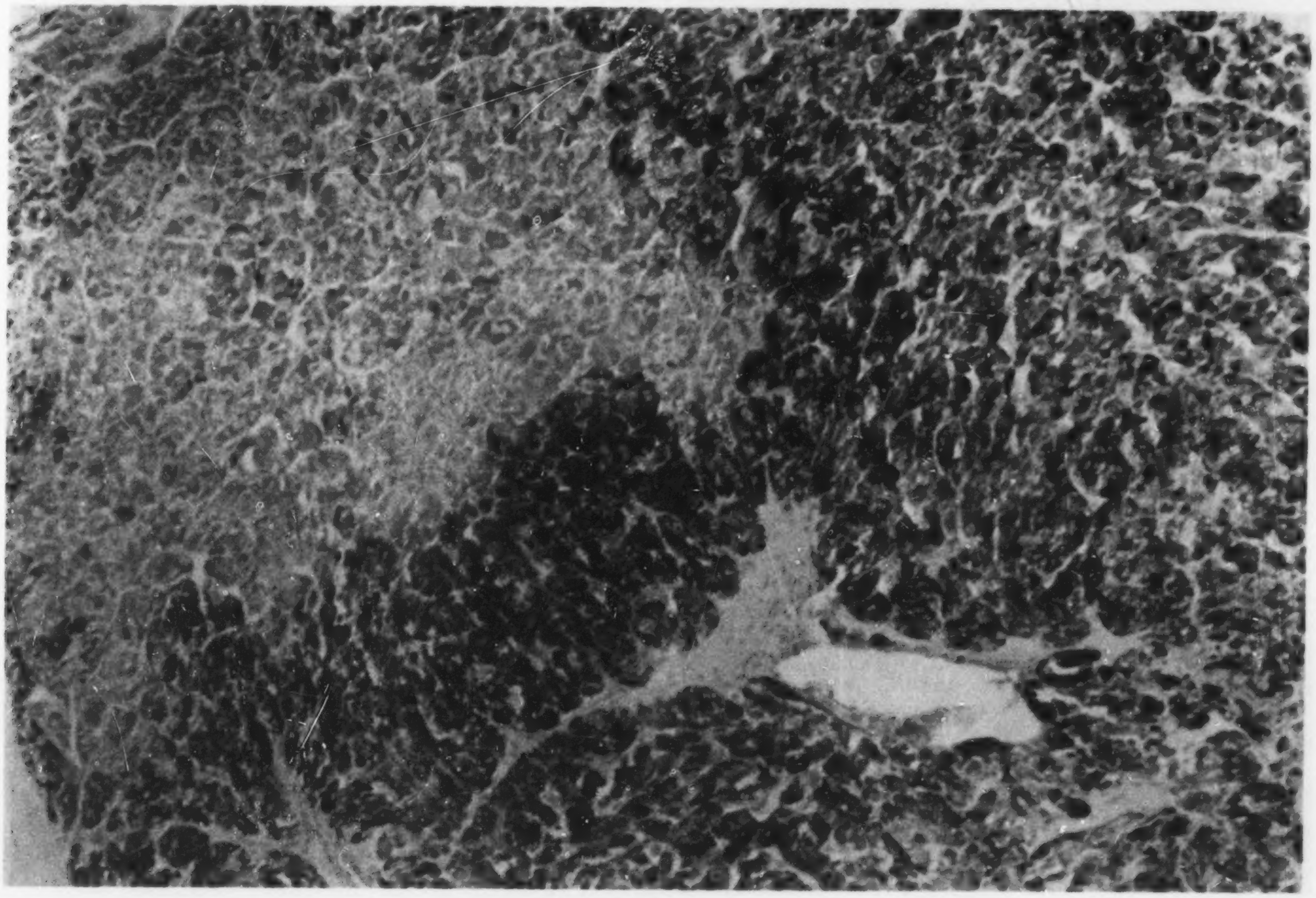
Dr. Geoffrey Mendelsohn of Johns Hopkins University: *"The question of what to do if the cancer is heterogeneous has not been answered."*



Heterogeneous cancer cells in the medulla of the thyroid gland: *the dark stain indicates the presence of the hormone calcitonin; the light stain indicates absence of the hormone. Such variety in tumor cells correlates with poor prognosis.*



CANCER CELLS *continued*

calcitonin-secreting cells in the thyroid mature, the investigators suggest that MTC cells may not mature completely.

A recent and as yet unpublished finding is that an inverse relationship exists between calcitonin and CEA (carcinoembryonic antigen), a marker present in several cancers, including those of the gastrointestinal tract, lung, and breast. According to preliminary results analyzed by Dr. Mendelsohn, CEA production may rise as calcitonin production drops. Cancer cells stained for both markers show almost no calcitonin when CEA is in high concentration.

"We're not really sure how to interpret these findings, but the going theory is that the high CEA content could result from failure of the cells to mature," says Dr. Mendelsohn. CEA is normally found in high concentrations in the developing fetus, particularly in the endodermal tissue. It is also present in the normal adult but only in trace quantities.

The researchers are now conducting molecular studies of animal and human cancer cells, hoping to uncover the biochemical reasons that these cells do not mature. "From previous work, it looks as if each tumor begins as a single cell; it has a clonal origin," says Dr. Baylin, "and as some tumors progress, heterogeneity develops within this clone of cells. Some patients develop little heterogeneity, while others develop an extreme biochemical difference in cell populations."

By working with Dr. Bernard Ross on tissue cultures from a rat model of medullary carcinoma and with Dr. Susan Leong on cultured cells from human MTC, Drs. Baylin and Mendelsohn are trying to get clones of cells that demonstrate this heterogeneity. Their intent is to study some of the factors that underlie failure of the cells to develop the biochemistry of their normal parent cells.

When Dr. Baylin began studying MTC in the mid-1970's, he teamed up with Dr. Samuel Wells. Both investigators were then at the National Institutes of Health. Dr. Baylin was interested in cancer cell development. Dr. Wells focused on diagnostic techniques.

The collaboration continued after Dr. Baylin left for Johns Hopkins and Dr. Wells joined Duke University, as director of the GCRC there. Both men treated families with a history of MTC and referred patients to each other. The collaboration will continue after Dr. Wells establishes his laboratory at the Washington University in St. Louis, where he is now chairman of the department of surgery. Dr. Wells also plans to continue studying the population of MTC subjects established at the Duke GCRC.

Drs. Baylin and Mendelsohn began studying the biochemistry of MTC some 5 years ago, when Dr. Mendelsohn was on a Hopkins fellowship in pathology and oncology. He has since joined the Hopkins faculty in pathology.

The discovery that MTC provides a unique model of how cancer cells develop was made largely by Drs. Baylin and Mendelsohn. Dr. Baylin applied the hypothesis of cancer cell progression from the homogeneous state to a mixed state. Dr. Mendelsohn characterized the tumor biochemically.

They have developed an increasingly precise delineation of MTC pathology and the description of a human cancer model that Dr. Baylin perceives as having great promise for examining events in human tumor cell progression. The research will affect diagnosis and treatment of MTC but, more important, it will give other researchers an example of how at least one type of cancer develops.

"Certainly animal models have demonstrated that tumor progression occurs and leads to the emergence of different cell populations over time," Dr. Baylin says. "Working with a human tumor model in which we can see and quantitate such cell changes, we should learn more about the implications of tumor progression and cell heterogeneity in human cancers."

Additional reading:

Lippman, S. M., Mendelsohn, G., Trump, D. L., Wells, S. A., Jr., and Baylin, S. B., The prognostic and biological significance of cellular heterogeneity in medullary thyroid carcinoma: a study of calcitonin, L-dopa decarboxylase, and histaminase. *Journal of Clinical Endocrinology and Metabolism* 54:233-240, 1982.

The Johns Hopkins University study of tumor composition and its effects on prognosis was supported by the National Cancer Institute. The current molecular study of MTC cells is funded by the American Cancer Society. The Johns Hopkins and Duke University General Clinical Research Centers are supported by the General Clinical Research Center Program of the NIH Division of Research Resources.

Pseudohermaphrodites: Models of the Developing Male

A disorder that masks the true sex of newborns has allowed researchers at Cornell University to study factors affecting the development of the male identity and to document the effects of male hormones. The disorder, called male pseudohermaphroditism, delays the appearance of male genitalia until puberty, when the production of testosterone increases.

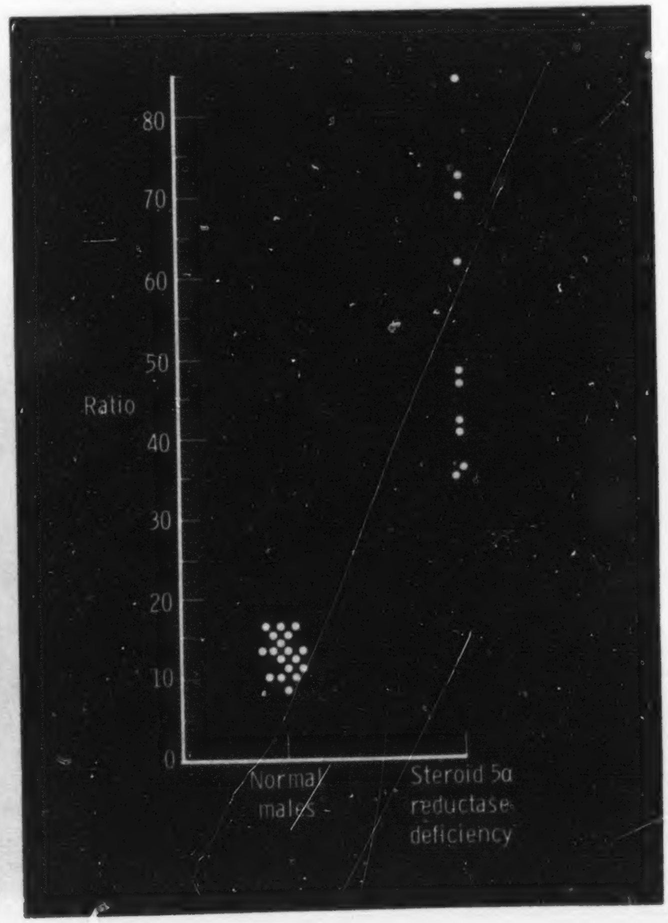
"It's the most natural experiment possible," says Dr. Julianne Imperato-McGinley, a Cornell biochemist. "These subjects are males with normal testes, making normal amounts of testosterone. Their brains were exposed to testosterone before birth and immediately after birth and then to high levels at puberty. Their hormonal profile is very similar to normal. But because of a biological trick—a mild enzyme deficiency—they are born with genitalia that appear to be female."

Dr. Ralph E. Peterson, a Cornell biochemist, explains that the cause of the disorder is a deficiency in the enzyme 5-alpha-reductase. The deficiency stops the conversion of testosterone to 5-alpha-dihydrotestosterone (DHT), the steroid mainly responsible for in utero development of male genitalia.

The condition may also occur due to a deficiency of the enzyme 17-beta-hydroxysteroid dehydrogenase. This deficiency has the same end effect, preventing the conversion of testosterone to dihydrotestosterone. "We have seen no subjects who completely lacked the enzyme. If there were a complete absence of

Dr. Julianne Imperato-McGinley of Cornell University, investigating male pseudohermaphroditism: *"The disease provides unique models for looking at the impact of testosterone in the developing male. This experiment of nature illustrates the importance of androgens in the evolution of male identity, both as inducers of the identity before and just after birth and as activators during puberty."*

Ratios of plasma testosterone to dihydrotestosterone in normal males and males with a deficiency of the enzyme 5-alpha-reductase: *insufficient quantity of the enzyme during gestation leads to male pseudohermaphroditism, a disorder that masks the true sex of newborns.*



HORMONES *continued*

enzyme, the genitalia would probably look entirely female," says Dr. Peterson, who documented the biochemistry of the disorder with Dr. Imperato-McGinley in the core laboratory of the Cornell University General Clinical Research Center (GCRC).

Children with either enzyme deficiency are born with a clitoris-like phallus, bifid scrotum, and urogenital sinus. The testes are in the inguinal canals or labial-scrotal folds. During puberty, testosterone production jumps about tenfold, allowing the male genes for external genitalia to be fully expressed. Male genitalia descend and grow to near-normal size, facial and body hair sprout, the voice deepens, and a characteristically male musculature develops. A masculine personality also develops until the manners and actions of these adolescents are clearly those of men.

Whereas DHT affects differentiation and growth of male external genitalia in utero, genital growth at puberty appears to be directly linked to testosterone, says Dr. Peterson. This explains why normal male structures develop after puberty in these patients and not in utero.

Affected males may have erections and ejaculations. Complete spermatogenesis was present on testicular biopsy in one subject, suggesting that testosterone may be more important in the regulation of spermatogenesis than DHT.

Plasma testosterone levels are normal or elevated in the adult subjects; DHT is low. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH), two major female hormones, are elevated, suggesting a role for DHT in the negative feedback control of LH and possibly FSH secretion.

Although the disorder has been reported rarely, cases have been identified in the United States, Jordan, France, England, Cyprus, North Africa, and New Guinea. By far the greatest incidence has been recorded in the Dominican Republic, a country of only 7 million people, where 34 cases have been found in recent years. Most were traced to common family roots.

Nineteen of the 34 island children were raised as girls because of their ambiguous genitalia at birth. These 19 children have now passed through puberty. Eighteen agreed to be studied physically and psychologically by the Cornell researchers. Of these, 16 had changed gender identity and gender role with the onset of puberty. They had developed male mannerisms, attitudes, and sexual tendencies, abandoning a childhood of rearing as females. According to Drs. Peterson and Imperato-McGinley, the emotional change occurs without prompting from parents or peers. In fact, the researchers say, it sometimes occurs despite the urging of society to maintain a female sex role.

The two village children in the study who did not completely change sex roles currently lead unconventional lives. One has changed to a male identity but continues to dress as a woman. His mannerisms are clearly masculine, according to Dr. Peterson, and he engages in sexual activity with village women. The other subject maintains the identity and role of a female. At

Dr. Ralph Peterson of Cornell University: ***"In most cases the decision to castrate a child is based on the appearance of external genitalia. If they look female, the physician's recommendation is castration."***

16 she married a man who left her after 1 year. She has since left the village and now lives alone, working on the island as a domestic servant. Despite a masculine build, she wears false breasts. She denies any attraction to women and plans to have castration in the hope that she can become a normal woman, according to Dr. Peterson.

He and Dr. Imperato-McGinley theorize that masculine behavior, even in those who maintain female gender identity, usually prevails because of sexual imprinting. They believe that testosterone often masculinizes the brain before and just after birth. The testosterone does not, however, cause male genitalia and male personality to develop fully until puberty, when production of the hormone increases greatly.

During puberty, the 16 subjects who assumed male identities felt a gradual change in their attitudes. Over several years they passed through stages in which they reported responses that progressed from "not feeling like girls," to "feeling like men," to the final conviction that they were indeed men.

On this basis, Drs. Imperato-McGinley and Peterson conclude that the effect of testosterone is more powerful than the influence of society, if the events of puberty are allowed to proceed normally. The word *if* is an important qualifier.

When ambiguous genitalia have been noticed at birth, castration has been the most common treatment in developed countries. Of the 11 reported cases in the United States, only 1 child was raised entirely as a boy; 1 child was reared first as a girl and then after 1 year, when his true sex was recognized, he was reared as a boy. Of the remaining 9 children, 7 were castrated before or during puberty. "In most cases the decision to castrate a child is based on the appearance of the external genitalia. If they look female, the physician's recommendation is castration," Dr. Peterson says.

Doctors in this situation fear that the genitalia will not develop into those of a normal male. Early castration and the start of lifelong female hormone therapy abort the physical change from female to male, but prenatal imprinting on the brain by male hormones may not be reversed.

The researchers just recently diagnosed the disorder in a woman who was castrated at age 5 and raised as a female. The castration didn't succeed in making her a woman. "She's 20 years old and she's an emotional mess," says Dr. Peterson. "She'll never be a normal female." He says the patient is unable to establish a clear gender identity. Her conflicting tendencies have produced emotional disturbances that affect her ability to function in society.

The Cornell doctors are currently dealing with an adolescent whose genitalia have only recently changed in appearance. Physical and emotional changes are still occurring, says Dr. Imperato-McGinley, but the mother is exerting serious pressure on the teenager to remain in a female role.

"Knowing what we do from the Dominican subjects, we think this person, in time, could completely change to have the body, attitudes, and mannerisms of a man, but the question is whether society—particularly the mother—will allow the transformation to occur," says Dr. Imperato-McGinley. "We really don't know what's going to happen."

Because Drs. Imperato-McGinley and Peterson have described the natural course of the anomaly, physicians now can be certain that the sexuality of children with the condition can be expected to develop normally. A rapid and accurate test developed by the Cornell biochemists assures proper diagnosis. The children can then be raised as boys.

If, however, diagnosis is not made until puberty, the children face embarrassment and possible harassment by their peers. "Some patients have a problem in changing gender role because a change would pose an embarrassing social problem for them and the family," Dr. Peterson says. "It's a social trauma in our

Early Diagnosis of Male Pseudohermaphroditism

Diagnosing male pseudohermaphroditism before puberty has been very difficult, researchers say. The genitalia are usually ambiguous. Doctors, not knowing that the abnormality is resolved at puberty, often "clarify" the child's sex by castrating him.

Dr. Ralph E. Peterson of Cornell University says castration can devastate the child. He believes that hormones masculinize the brain during fetal development, causing a male personality to evolve despite the absence of male genitalia. The result can be serious emotional disturbances that appear later in life.

Now a quick and effective method has been devised for diagnosing the disorder. It relies on a gas chromatography technique available at the Biomedical, Bio-Organic Mass Spectrometry Resource at the University of California at Berkeley.

In testing for the disease, Dr. Cedric Shackleton, a biochemist at the resource, and Drs. Peterson and Julianne Imperato-McGinley of Cornell University Medical School measure the levels of several urinary steroids produced by the enzymes 5-beta-reductase and 5-alpha-reductase. According to the researchers, male pseudohermaphrodites with 5-alpha-reductase deficiency have a marked reduction in the concentration of 5-alpha-dihydrotestosterone in blood plasma. These patients produce a large amount of etiocholanolone, an inactive urinary 5-beta metabolite of testosterone, and produce only a little androsterone, the 5-alpha metabolite. If the disorder is present, the ratio of 5-beta-tetrahydrocortisol to 5-alpha-tetrahydrocortisol is markedly increased at 2:5. The normal ratio is 1:4. "We hope that this method will complement conventional diagnostic techniques, which often are lengthy, complicated, and nonspecific," says Dr. Peterson.

The University of California mass spectrometry resource is one of the few facilities in the United States that can provide detailed gas chromatographic analysis of urinary steroids for clinical diagnosis. By using advanced techniques in gas chromatography, such as rugged, fused silica capillary columns instead of conventional glass columns and $BaCO_3$ column-coating techniques, the researchers can separate mixtures at temperatures as high as 340°C. With these techniques, steroids and other heavy compounds can be identified and quantified.

Since the mass spectrometry resource was established in 1979, Dr. Shackleton and his associates, in collaboration with other investigators, have identified hundreds of cases of endocrine disorders. The diagnostic service is available free to investigators who have already conducted complete medical examinations of patients but are unable to confirm a disorder using conventional diagnostic techniques.

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Shackleton, C. H. L., Urinary steroid metabolite analysis: potential for renaissance. *Clinical Chemistry* 27:509-511, 1981.

The Biomedical, Bio-Organic Mass Spectrometry Resource at Berkeley is supported by the NIH Division of Research Resources.

society only in the sense that peers tend to make fun of the role changers."

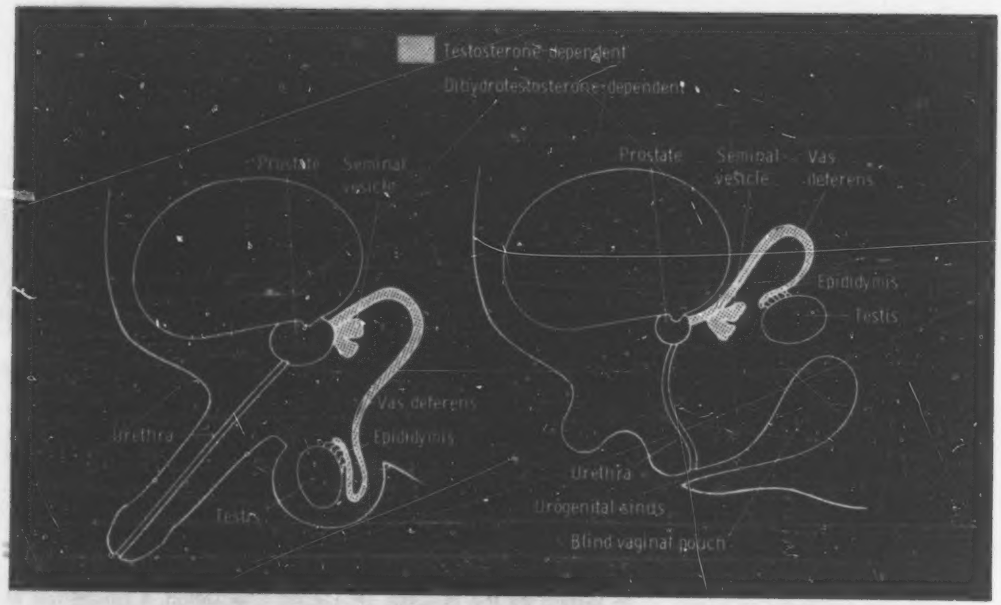
This fear of embarrassment and ridicule may be overpowering. One of the two American children who was not castrated has maintained a female identity, despite the development of male genitalia. Those in the Dominican Republic who have changed to a male identity report anxiety about entering into sexual relationships. They fear ridicule, says Dr. Imperato-McGinley. Many see themselves as incomplete. None, however, shows serious emotional damage. "They have suffered, but they are normal—impressively normal," says Dr. Imperato-McGinley.

Such is the case of the other American child who was not castrated. Despite an emotionally traumatic childhood, he developed into a normal adult male. At age 12 the child entered puberty and developed all the physical characteristics of a male. As embarrassment over his appearance grew, the child became increasingly unhappy, made few friends, barely obtained passing grades, and finally dropped out of school.

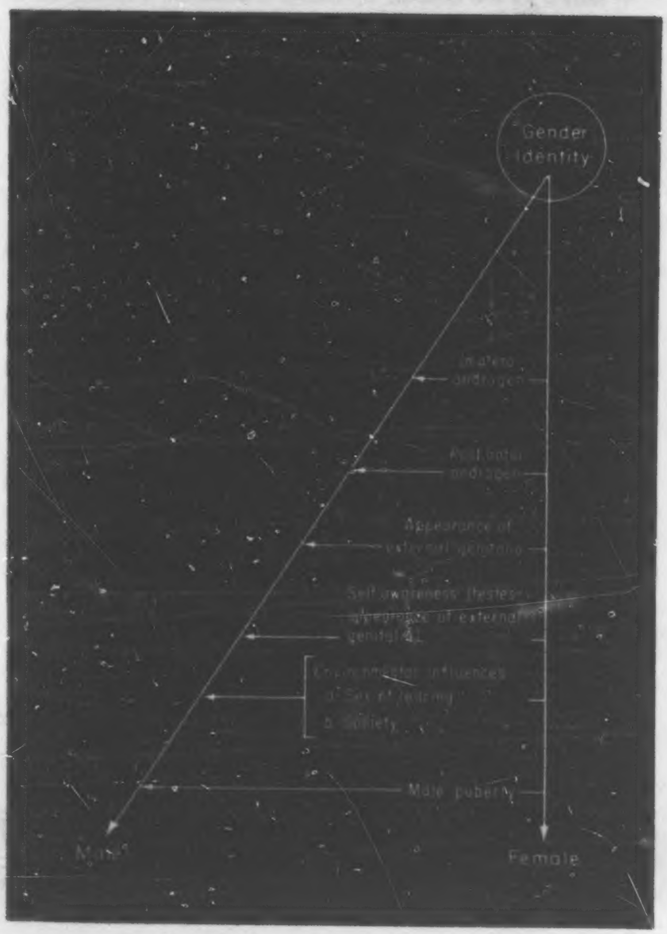
Medical evaluation and the decision to change sex roles vastly improved the teenager's disposition. It was as though he finally understood why he looked and behaved as he did. He changed to wearing boy's clothing, got a boy's haircut, and thereafter looked and behaved like a normal boy, according to Dr. Peterson.

The family moved to a new neighborhood. The child joined a new high school, became an athlete, began dating girls, and excelled as a student, particularly in mathematics, a subject in which he had done very poorly before. He finished high school, college, and postgraduate work. At 25 he married. The couple now report a mutually satisfactory sex life.

Some men with the condition choose to tread a middle ground between male and female identities. One such case is a 66-year-old immigrant from Italy. As a


Testosterone dependent
Dihydrotestosterone dependent
Prostate
Seminal vesicle
Vas deferens
Epididymis
Urethra
Testis
Prostate
Seminal vesicle
Vas deferens
Epididymis
Testis
Urethra
Urogenital sinus
Blind vaginal pouch

The roles of testosterone and dihydrotestosterone (DHT) in male sexual differentiation before birth: *insufficient levels of DHT delay complete development of male genitalia until puborty.*


Gender Identity
In utero androgen
Post natal androgen
Appearance of external genitalia
Self awareness (testes-appearance of external genitalia)
Environmental influences of sex of rearing & society
Male puberty
Male
Female

Critical factors in the evolution of male gender identity in humans: *judging the influence of biology and rearing.*

child he was considered a tomboy, played ball, pitched pennies, and rode burros with boys. He rarely played with dolls. Between ages 13 and 15, his voice deepened, facial hair developed, and male genitalia grew. Soon erections and ejaculations were possible.

Since his late teen years, he has had numerous sexual experiences with women but has continued to conceal his male identity from his family. After his father's death, he lived with and supported his mother who died last year at age 94.

"He had planned to change to a male role when his mother died, but I don't think that's going to happen," Dr. Peterson says. "He's nearly 70 years old now, and he still has brothers, sisters, nephews, and nieces. What is he going to tell them? That he's an uncle, not an aunt?"

Last year the researchers interviewed him. He wore a tailored pant suit but no makeup or jewelry. He had a deep voice, a man's style haircut, and a masculine manner. He recalled his shock when male rather than female features appeared at puberty. Since puberty he has felt like a male, he says, but because of family and social pressures, he has continued to present himself publicly as a female.

"The gender change has occurred. The patient is clearly male. He even functions sexually as a man, and he feels like a man," says Dr. Imperato-McGinley. "But the social and particularly family settings have prevented a complete change socially and legally. He prefers to masquerade as a female rather than change completely to assume a male sex role."

The patient did, however, undergo minor surgery 2 years ago to correct two anomalies of the disorder—undescended testes and mild chordee, a downward curvature of the penis. Other patients have requested and undergone similar operations. All have convalesced following surgery in the Cornell GCRC.

Although outweighed by the psychological trauma of the condition, male pseudohermaphroditism does offer certain physiological advantages. A receding hairline or hair loss has never been reported by people with the disorder and acne does not occur, despite family patterns to the contrary. The Cornell doctors speculate that the low level of DHT present throughout life may be a factor in these characteristics.

The major finding by the Cornell researchers, however, relates directly to the development of gender identity. "The disease provides unique models for looking at the impact of testosterone in the developing male," says Dr. Imperato-McGinley. "This experiment of nature illustrates the importance of androgens in the evolution of male identity, both as inducers of the identity before and just after birth and as activators during puberty."

From their observations, the Cornell investigators conclude that hormones play the central role in determining sexual identity, although culture exerts a powerful and sometimes overwhelming force. "If natural events proceed unchallenged, biology exerts the determining force in gender identity, despite years of rearing to the contrary," says Dr. Imperato-McGinley. "But in a rigid society, where change is not well accepted, gender identity can be imposed on people despite biological forces." **R**

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Funding for the Cornell study of pseudohermaphroditism is provided by the National Institute for Child Health and Human Development. The General Clinical Research Center (GCRC) at Cornell University is supported by the GCRC Program of the NIH Division of Research Resources.

Research Focus

Lithium Studies Examine Manic-Depressive Illness

By documenting the biological effects of lithium, a drug commonly prescribed for manic-depressives, investigators are attempting to explain the mechanism of the disease and to improve treatment.

Using mass spectrometry instruments at the Washington University School of Medicine in St. Louis, researchers are following one of the metabolic changes associated with lithium administration to rats. Dr. William Sherman, professor of biochemistry in the university's psychiatry department, has evidence that lipid metabolism in the brain is altered in rats given pharmacologically effective doses of lithium. According to research by Dr. Sherman and his colleagues, lithium changes the metabolism of a group of lipids called phosphoinositides. These lipids appear to be involved in synaptic transmission, receptor activation, and hormonal release in the brain. They are known to be metabolically stimulated by cholinergic neurotransmitters in the nervous system. When atropine, a drug that inhibits the acetylcholine-mediated neuropathway in the brain, was administered, lithium's action on the rat brain was completely inhibited.

"These findings suggest that lithium stimulates the cholinergic pathway with simultaneous alteration of phosphoinositide metabolism," says Dr. Sherman. The metabolism of phosphoinositides involves a chain of chemical reactions, one step of which is the conversion of *myo*-inositol-1-phosphate to *myo*-inositol. Dr. Sherman found that part of lithium's effect on the brain is to block the enzyme that catalyzes that conversion.

Following research pioneered by the late Dr. James H. Allison, Dr. Sherman administered lithium once a day to rats for 9 days to establish which substances were altered and investigated which layers of the cerebral cortex were affected by lithium. The drug caused a 30 percent decrease in levels of *myo*-inositol throughout the cortex, but the cerebellum was unaffected. At the same time, *myo*-inositol-1-phosphate (M1P) was found to be 20 to 50 times above control levels.

"Because the full effect of lithium treatment is not observed until 1 to 2 weeks after it is first

Dr. William Sherman at the Washington University School of Medicine: *examining lithium's effect on lipid metabolism in the brain.*

administered, we believe that some metabolic process must be gradually changed in the central nervous system during this time," says Dr. Sherman. "Since the effect that we are studying occurs immediately upon administration of lithium, it is probably not the process that is ultimately altered in the recovery from mania. However, since phosphoinositide metabolism is very important in nerve tissue, it may be that prolonged alteration of the lipid metabolism ultimately effects a change in those processes which are responsible for mania." He hopes to learn if this effect occurs only in the brain or if lithium changes lipid metabolism in other parts of the body.

Dr. Peter Stokes, associate professor of psychiatry and medicine at the New York Hospital-Cornell University Medical Center, is searching for ways to improve lithium treatment by determining the cause of its toxicity. Recently, lithium treatment has been associated with irreversible kidney damage in some patients. Routine dosages of lithium suddenly can become highly toxic if a patient loses too much fluid due to excessive sweating, restricted sodium intake, or vomiting and diarrhea caused by illness.

Dr. Stokes has found that the toxic effects that occur in some patients may be related to the metabolic properties of one of lithium's two isotopes. Natural lithium exists as two isotopes: mass 7 (Li-7) and mass 6 (Li-6). Dr. Stokes believes that Li-6 may be more responsible than Li-7 for causing toxic symptoms in some patients. In association with Drs. Kenneth Lieberman and George Alexander, he found in studies involving both low and high doses of lithium isotopes that Li-6 produced more side effects and resulted in more deaths than equal doses of Li-7 in rats, mice, and cats.

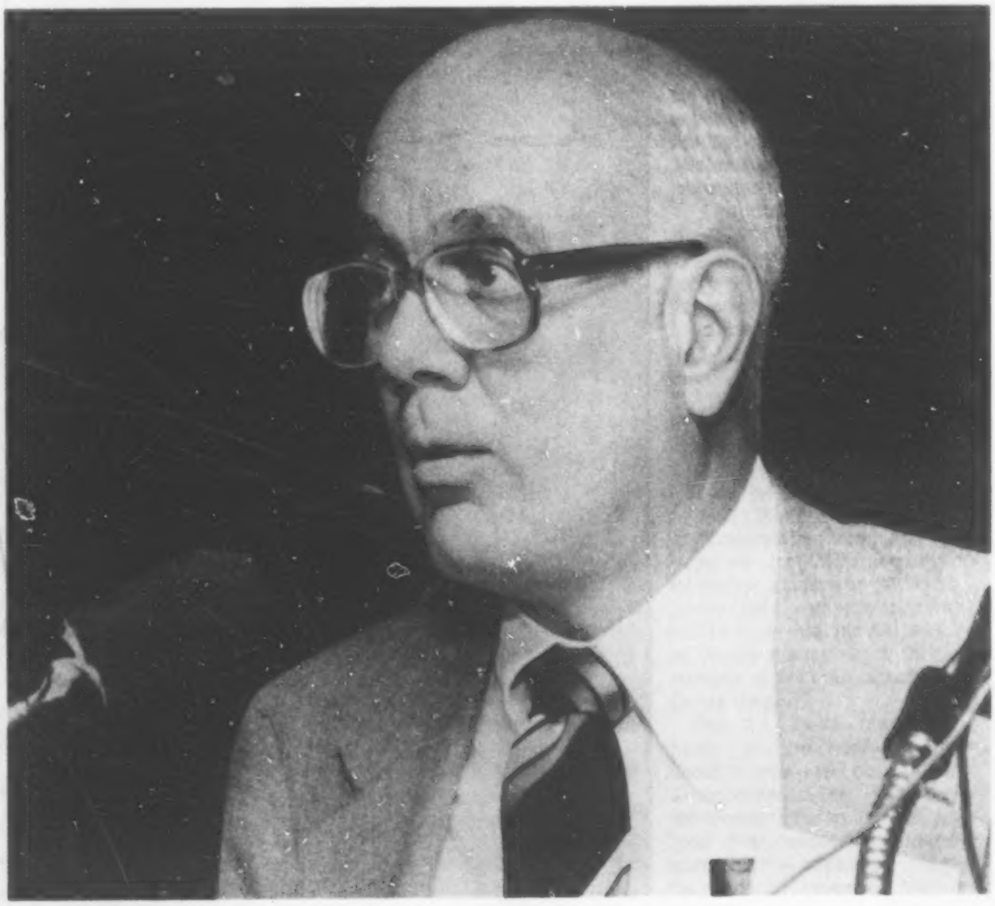
"Our studies are probably the first to demonstrate that isotopes have different pharmacokinetic properties in living systems," says Dr. Stokes. Traditionally, different isotopes of the same element have been believed to differ in their rate of reaction but not in their metabolic properties.

Using the mass spectrometry resource at Rockefeller University to characterize samples, Dr. Stokes prepared quantities of Li-6 and Li-7 isotopes for animal studies. He observed that rats treated for 5 days with low doses of Li-6 became less mobile, lost significantly more weight, and urinated quantities 10 times greater than rats treated with Li-7. The onset and severity of effects from Li-6 were more rapid and more profound than those from Li-7. Eight of 10 rats treated with Li-6 died, compared to 1 of 10 rats treated with Li-7. No control rats, which had been given water, died.

Dr. Stokes believes that Li-6 is more toxic than Li-7 because it is transported across cell membranes at a faster rate. In early studies he demonstrated that human red blood cells, in vitro, take up 6 to 8 percent greater quantities of Li-6 than Li-7. His studies included measurements from subjects who were psychiatrically normal, from manic-depressives, and from primary alcoholics.

"Our work suggests that the molecular weight of elements may determine their metabolic distribution throughout the body," he says. "Isotopes of lower molecular weight may be transported across various membranes at a faster rate than their heavier counterparts and may, thereby, exert different physiological properties."

Working with Dr. Michiko Okamoto, he found in cats that Li-6 entered plasma and cerebrospinal fluid at a faster rate than Li-7. He obtained further evidence that Li-6 is more toxic by relating the behavior of the cats to the concentration of Li-6 as it moved from plasma to the central nervous system. Cats treated with both Li-6 and Li-7 became lethargic and consequently immobile immediately after a large single dose was administered. The cats later developed diarrhea and sweated and urinated excessively. Within 24 hours the behavior of both groups appeared to improve and the cats appeared to have partially re-



Dr. Peter Stokes of the New York Hospital at Cornell University Medical Center: ***searching for ways to improve lithium treatment by determining the cause of its toxicity.***

covered. This behavior corresponded to decreases in plasma levels of Li-6 and Li-7. After 2 to 3 days, levels of Li-6 and Li-7 increased substantially in the cerebrospinal fluid, as if the lithium had moved from the blood to the brain, says Dr. Stokes. During this time, the Li-6-treated cats again became lethargic and quiet, while Li-7-treated cats fully recovered. Most cats treated with Li-6 died. Dr. Stokes says the accumulation of Li-6 in the central nervous system apparently caused fatal neurological damage.

Dr. Stokes currently is testing patients with manic-depressive illness to determine if Li-7 improves behavior as effectively as the clinically used drug, which contains both isotopes. Preliminary results indicate the Li-7 is as effective therapeutically as the conventional drug, he says.

"Eliminating Li-6 from the drug may increase safety. If future studies confirm that Li-7 is as therapeutic as the clinically used drug, only Li-7 should be prescribed," says Dr. Stokes.

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Dr. Sherman's research into phosphoinositides is supported by the National Institute of Mental Health and the National Institute of Neurological and Communicative Disorders and Stroke. The Resource for Biomedical Mass Spectrometry at the Washington University School of Medicine is supported by the Biotechnology Resources Program (BRP) of the NIH Division of Research Resources. Dr. Stokes' research into the metabolic differences of lithium isotopes is supported by a gift from Mr. and Mrs. Paul Hexter and the National Institute of Mental Health. The Mass Spectrometric Biotechnology Resource at the Rockefeller University in New York is supported by the BRP.

Illustration by Thomas Rowlandson (18th century) of a patient with symptoms characteristic of manic-depressive illness: *understanding the biochemical effects of lithium, a drug used to treat the illness, may explain the mechanisms of the disease and lead to improved treatment.*

Illustration courtesy National Library of Medicine

Research Resources Reporter
May 1982

Information Exchange

Research Highlights

Simple meditative practices that elicit the relaxation response mimic the beneficial effects of certain drugs widely prescribed for high blood pressure, cardiac irregularities, migraine headaches, and anxiety, according to research done at the Beth Israel Hospital General Clinical Research Center in Boston.

"This research helps explain why the calming effects of the relaxation response are carried over into other periods of the day, protecting the meditator from 'overstress,'" says Dr. John W. Hoffman, a member of the research team.

Scientists have known for years that simple meditative practices are useful in relieving stress and calming the body, but they have not fully understood why. (See *Research Resources Reporter*, August 1981, pages 11-12.) They now know that the relaxation response, like drugs prescribed for hypertension, cardiac irregularities, migraine headaches, and anxiety, blocks the effects of epinephrine and norepinephrine, hormones that are commonly secreted when the brain perceives danger.

In times of stress these hormones cause heart rate to increase, blood pressure to rise, breathing to become more rapid, and muscle tension to increase. This emergency response was first identified by Harvard physiologist Dr. Walter B. Cannon in the early 20th century and was dubbed the fight-or-flight response.

"But each of us possesses a natural and innate protective mechanism against 'overstress,' which allows us to turn off harmful body effects to counter the effect of the fight-or-flight response," says Dr. Herbert Benson, head of the Harvard behavioral medicine division and associate professor of medicine at Harvard Medical School. He has labeled this mechanism the relaxation response.

This response is a set of specific physiological changes that are elicited by meditation-relaxation techniques. Although they have existed for centuries in certain practices of Eastern cultures, the physiological effects have only recently been identified and investigated.

According to Dr. Benson, four elements are needed to elicit the relaxation response: a quiet environment, a comfortable position, a passive attitude toward intervening thoughts, and most important, the repetition of a word, prayer, sound, or phrase.

When practicing the relaxation response, predictable body changes occur, which are the opposite of those resulting from the fight-or-flight response, says Dr. Benson. The emergency response, characterized by increased secretion of norepinephrine, also called noradrenalin, leads to increased heart rate, high blood pressure, faster breathing, and increased skeletal blood flow. The relaxation response, in contrast, decreases blood pressure, reduces the heart rate by about three beats per minute, lowers oxygen consumption by 10 to 20 percent, stabilizes blood flow to the muscles, slows body metabolism, and increases alpha brain waves, which are associated with a feeling of relaxation and well-being.

What has been puzzling to researchers is why, in people who practice the relaxation response, the norepinephrine in the blood is not decreased as are blood pressure and heart rate.

"What we found to our surprise was that under stress there was actually more norepinephrine secreted into the bloodstream in people who meditated—exactly the opposite of what we had expected," says Dr. Hoffman. "But despite more norepinephrine being secreted, blood pressure and heart rate did not increase as in the case of the fight-or-flight response."

The study showed that, although more norepinephrine is secreted in people who meditate, the hormone is blocked from acting and perhaps is unable to bind to the organs where it has its effects. Although the reasons for this are unclear, it explains why practicing the relaxation response twice daily for 10 to 20 minutes protects against "overstress" by altering the body's responses to norepinephrine.

This does not mean, however, that when the body needs more norepinephrine to respond to an emergency, the hormone's action will be completely blocked. In fact, when the study subjects stood up after lying down, the stress of suddenly standing caused their blood pressure and heart rate to rise—a result of more norepinephrine reaching the organs. This means that a person who meditates remains calmer throughout the day because of this altered responsivity to norepinephrine, yet when the body needs more of the hormone, it becomes receptive to it.

Several drugs used in the treatment of high blood pressure act in a similar way, preventing the effects of norepinephrine. Yet the relaxation response brings about the same result naturally. Dr. Benson cautions, however, that the relaxation response should not be used to replace prescribed medication. "It may be used in conjunction with drugs but not as a substitute," he says.

This combination of approaches is currently being applied at Beth Israel in the hypertension program of the behavioral medicine division. The 6-month program, under the direction of Dr. Margaret Caudill, emphasizes the nonpharmacologic treatment of hypertension. It includes the teaching of the relaxation response, salt and calorie restriction, and exercise.

"The program introduces specific tools that will enhance the body's innate capacities to return to homeostasis or normal balance," says Dr. Caudill. "In addition to lowering blood pressure, individuals who regularly practice the techniques experience a sense of well-being."

Participants in the study, in addition to Drs. Hoffman, Benson, and Caudill, include Drs. Lewis Landsberg, Gene Stainbrook, James Young, and Andrew Gill, and Ms. Patricia Arns.

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Study of the relaxation response and the similarity between its effects and those produced by widely prescribed drugs is supported by the National Heart, Lung, and Blood Institute. The Beth Israel General Clinical Research Center (GCRC) is funded by the GCRC Program of the NIH Division of Research Resources.

The first nuclear magnetic resonance (NMR) spectrometer in the United States based on a persistent, or stable, superconducting magnet will be built for Stanford University under a contract with Japan Electron Optical Laboratories.

"The 600-MHz spectrometer will provide to the scientific community the most powerful instrument of its kind," says Dr. Oleg Jardetzky, professor of pharmacology and director of the Stanford Magnetic Resonance Laboratory.

A similarly powerful spectrometer was constructed in 1978 at the Carnegie-Mellon University in Pittsburgh. (See *Research Resources Reporter*, April 1978, page 6.) It is based, however, on a nonpersistent magnet, which requires frequent recharging. Persistent magnets with high-power field and high resolution of the type that will be installed at Stanford were not technically feasible at the time, according to Dr. Jardetzky.

The 14 Tesla magnet—the heart of the high-resolution spectrometer—will be built under a subcontract by Oxford Instruments, Ltd., in Oxford, England. The company is currently building the prototype magnet for Oxford University. Delivery of the new spectrometer system at Stanford is scheduled for the spring of 1983.

Work at the Stanford Magnetic Resonance Laboratory is devoted to the study of biological structure and function and to the development of possible applications to medical diagnosis, Dr. Jardetzky explains. "There are many ways to look at matter and nature up close," he says. "There are conventional optical microscopes, powerful electron microscopes, X-ray diffraction methods, and a whole family of spectrometers. The NMR spectrometer is one of the most powerful of these devices in that it allows scientists to 'look' at structures many times smaller than can be seen with even the best electron microscope."

NMR makes it possible for researchers to pinpoint all the atoms in a complex protein molecule. Moreover, it allows them to observe exactly how the atoms move through space and over time. For example, a scientist may label amino acids—the building blocks of proteins—with stable isotopes, and from NMR spectra he can chart changes in protein structure that result from their interaction with drugs or other chemical agents.

The studies aid in understanding disease; for example, NMR can be used to analyze abnormally shaped proteins associated with hereditary disease.

The NMR spectra can be displayed on a video screen every few seconds, and the memory of each spectrum can then be stored in a computer, allowing a scientist to follow changes in molecular shape and motion.

"We can infer many things about the dynamic movement of these molecules," Dr. Jardetzky says. "And we are in a position to develop a model of a protein as it is working."

Other methods of looking at proteins, he says, require that the molecules be frozen for viewing. The NMR method, however, allows them to float freely in solution and remain biologically active.

The Stanford laboratory, Dr. Jardetzky says, previously pioneered the development of a 360-MHz NMR spectrometer, which at the time of its installation in 1974 was the most powerful instrument available anywhere. "Significant advances in our understanding of the structure and dynamics of proteins, nucleic acids, and membranes have resulted from the experiments done on that spectrometer," he says.

Over the past 2 years, applications of NMR at the laboratory have also been extended to living systems, making measurements inside plant and animal cells without destroying, invading, or otherwise disturbing the organism.

"The new, 600-MHz instrument will allow much better resolution of spectral lines," Dr. Jardetzky adds, "and, therefore, still more detailed examination of molecular structures and cell constituents than has been possible so far."

The new NMR spectrometer is being financed by a grant from the Biotechnology Resources Program (BRP) of the NIH Division of Research Resources. The Stanford Magnetic Resonance Laboratory operates as a national resource and is supported by the BRP.

Research Service

Marmoset Resource

A new Marmoset Colony for Biomedical Research, at Texas A&M University in College Station will soon provide live marmosets, particularly *Callithrix jacchus*. It is supported by the Animal Resources Program of the NIH Division of Research Resources.

Guest investigators, collaborating scientists, and graduate students may utilize animals in the closed colony for studies not injurious to the animals' health or reproductive efficiency. Animals are available especially for projects in the area of behavioral research.

Other services provided by the resource on a fee basis are housing and animal care; assistance in animal manipulation and access to a procedural room; and clinical pathology and histopathology analyses and serum, tissue, and organ collection. The core staff also provides general consulting in veterinary anatomy, microbiology, parasitology, physiology, pharmacology, public health, biochemistry, biophysics, human anatomy, and dental research. Outside users of the resource must provide their own funding.

Fees for serum, tissues, organs, and preserved whole animals, now available, are currently negotiable. Live animals, when available, will cost between $350 and $550 each. Animals in the colony have been characterized hematologically, biochemically, and microbiologically.

Marmosets have a number of characteristics that make them valuable research animals, according to Drs. Raymond F. Sis and Roscoe W. Lewis, coprincipal investigators at the resource. They are smaller, more economical, and easier to handle than other primates, and yet they are large enough for routine surgical procedures. The size range varies from the pygmy marmoset, weighing only 130 to 150 grams, to the larger species, which are about the size of a squirrel. The cost for upkeep and maintenance is about the same as the cost for the cat.

Marmosets are sexually mature at about 17 months of age. They have a short gestation period and produce an average of three surviving young per year. "Twinning is the rule with marmosets," Dr. Sis says, "and most twins are chimeric; that is, they contain cells of different origin such as blood cells derived from a dizygotic co-twin. Chimerism is a factor found in no other research primates. Triplets are also

Marmoset twins at the Marmoset Colony for Biomedical Research: ***smaller, more economical, and easier to handle than other primates, marmosets are still large enough for routine surgical procedures.***

common, and since the mother is unable to feed all the offspring at one time, the resource staff has developed a procedure to hand-feed the infants."

The resource currently has approximately 85 breeding pairs of *Callithrix jacchus*. These South American primates are maintained at indoor/outdoor facilities, which provide a controlled-temperature room for eating and sleeping and a simulated natural habitat outdoors for exercise.

Marmosets, especially *Callithrix jacchus*, are used in dental research because the development of their teeth as well as the number, kind, and arrangement are similar to those of man. Moreover, they spontaneously develop a chronic destructive periodontitis, although they are relatively free from caries.

Callithrix jacchus is also a valuable animal for teratology research. Its short gestation period and relatively large number of offspring make it well suited for studies on congenital defects, and it is also valuable in behavior and reproduction research.

The resource staff is currently conducting research in several different areas. A major project of international importance is focused on the wasting marmoset syndrome, which is of unknown etiology. Nutrition, malabsorption, congenital defects, and behavior are other research areas being studied in-house. Dr. Sis is particularly interested in the possibility of developing the marmoset as a model for human nutrition.

Inquiries are welcomed from investigators who would like to obtain marmosets for their research. Requests are reviewed and assigned priorities by the animal use committee.

For further information about the resource and its services, contact:

Dr. Frank Stein
Department of Veterinary
Anatomy
Texas A&M University
College Station, Texas 77843
Telephone (713) 845-2828

Poultry Registry Available

The fourth edition of the *Poultry Registry of Genetic Stocks* has been published and is available to investigators who use poultry in their scientific research.

The book describes known genetic traits, breeds and varieties, specialized lines and strains, and mutations of chickens, Japanese quail, and turkeys from 17 countries. The directory also describes chicken plumage colors grouped according to the E locus and contains three chromosome linkage maps and addresses of breeders and suppliers.

Copies are available at $3 each from Dr. Ralph G. Somes, Jr., Department of Nutritional Sciences, University of Connecticut, Storrs, Connecticut 06268.

Meetings

Nuclear Accelerator Conference

The Seventh Conference on the Application of Accelerators in Research and Industry will be held from November 8 to 10, 1982, at North Texas State University, Denton. Physicists and other scientists will discuss production of radioisotopes for use in medicine, radiation therapy for cancer treatment, design of machines for medical applications, theoretical and experimental aspects of nuclear and atomic phenomena, and problems related to fusion power generation. The deadline for abstracts is July 1, 1982.

For further information, contact:

Dr. Jerome L. Duggan
Department of Physics
North Texas State University
Denton, Texas 76203
Telephone (817) 788-2626

June 1-3, 1982

Title: *9th Annual Symposium on Recent Advances and Common Problems in Pediatrics: A Small Group Approach*
Location: *Washington, D.C.*
Contact: *Susan Weiss*
Convention Services
Children's Hospital National Medical Center
111 Michigan Avenue, N.W.
Washington, D.C. 20010
Telephone (202) 745-3000

June 2-4, 1982

Title: *Molecular Biology of Fibrinogen and Fibrin*
Location: *New York, New York*
Contact: *Conference Department*
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021
Telephone (212) 838-0230

June 6-11, 1982

Title: *International Symposium on the Synthesis and Applications of Isotopically Labelled Compounds*
Location: *Kansas City, Missouri*
Contact: *A. Susan*
Scientific Secretary of the Symposium
c/o Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110
Telephone (816) 753-7600

June 10-12, 1982

Title: *5th International Symposium on Brain Edema*
Location: *Groningen, The Netherlands*
Contact: *Symposium Secretariat*
P.O. Box 252
6710 BG EDE
The Netherlands

June 13-16, 1982

Title: *The 1982 Birth Defects Conference*
Location: *Birmingham, Alabama*
Contact: *Dr. Wayne H. Finley*
Director
Laboratory of Medical Genetics
University of Alabama
University Station
Birmingham, Alabama 35294
Telephone (205) 934-4968

June 14-17, 1982

Title: *8th International Convocation on Immunology*
Location: *Amherst, New York*
Contact: *Dr. James F. Mohn*
Director
The Ernest Witebsky Center for Immunology
Room 210, Sherman Hall
State University of New York at Buffalo
Buffalo, New York 14214
Telephone (716) 831-2848

June 14-19, 1982

Title: *World Congress of Gastroenterology, Digestive Endoscopy, and Coloproctology*
Location: *Stockholm, Sweden*
Contact: *Stockholm Convention Bureau*
Jakobs Tong 3
S-111 52 Stockholm, Sweden

June 17-18, 1982

Title: *International Symposium on Calcium Modulators*
Location: *Venice, Italy*
Contact: *Secretariat*
Fondazione Giovanni Lorenzini
Via Monte Napoleone 23
20121 Milan, Italy

June 20-23, 1982

Title: *International Conference on Chromatography and Mass Spectrometry in Biomedical Sciences*
Location: *Bordighera, Italy*
Contact: *Dr. Alberto Frigerio*
Gruppo Italiano di Spettrometria di Massa in Biochimica e Medicina
Via Eritrea 62
20157 Milano, Italy

June 21-24, 1982

Title: *21st Annual Convention of the Canadian Association for Laboratory Animal Science*
Location: *Saskatchewan, Canada*

Contact: *Dr. Ernest D. Olfert*
Director
Animal Resources Center
University of Saskatchewan
Saskatoon, Saskatchewan
Canada S7N OWO

June 21-25, 1982

Title: *2nd International Symposium on Infant Nutrition and the Development of the Gastrointestinal Tract*
Location: *Niagara Falls, Ontario, Canada*
Contact: *Rayna Dutton*
Pediatric Continuing Medical Education
Children's Hospital
219 Bryant Street
Buffalo, New York 14222
Telephone (716) 878-7630

June 28-July 2, 1982

Title: *14th Michigan Molecular Institute Symposium: The Extracellular Matrix*
Location: *Midland, Michigan*
Contact: *Symposium Secretary*
Michigan Molecular Institute
1910 West St. Andrews Road
Midland, Michigan 48640
Telephone (517) 631-9450

July 12-14, 1982

Title: *4th PRI International Conference on Plastic in Medicine and Surgery*
Location: *Durham, United Kingdom*
Contact: *K. Copeland*
Biological Engineering Society
Royal College of Surgeons
Lincoln's Inn Fields
London WC2A 3PN,
United Kingdom

July 12-16, 1982

Title: *International Conference and Exhibition on Microscopy*
Location: *London, England*
Contact: *Royal Microscopical Society*
37/38 St. Clements
Oxford OX4 1AJ, England

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Research Resources Reporter

June 1982, Volume VI, No. 6

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This Issue:

Beads Deliver Drugs
PROPHET
Attitudes Toward AI
HVEM Resource

by
Donald W. McKinstry, Ph.D.

Diagnosis, Cause, and Treatment Of Alzheimer's Disease

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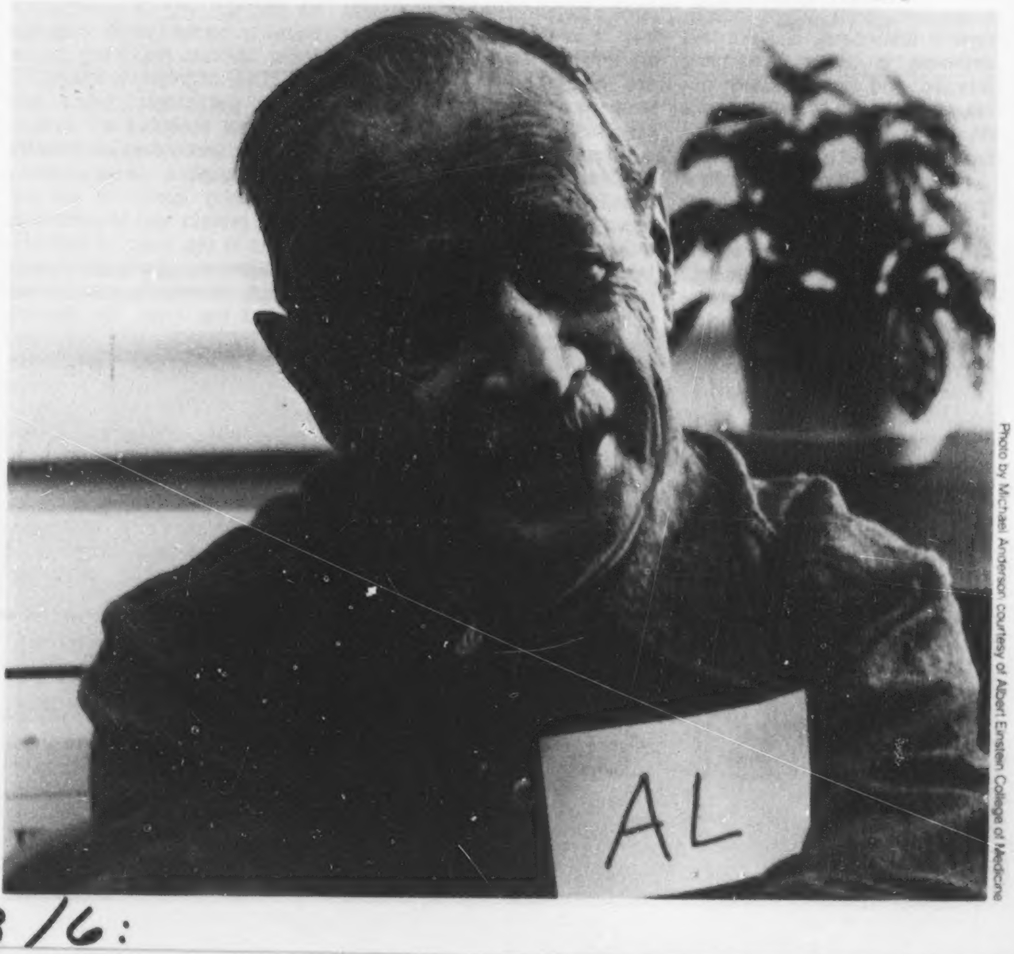
Drugs and dietary supplements are being studied at a number of medical centers both here and abroad for possible value in treating Alzheimer's disease, which causes a progressive mental decline in middle-to-late life. There is no cure for the disease, although several therapies show promise for reducing, at least temporarily, the memory and learning impairments associated with the disorder. Moreover, recent pathologic and pathogenic findings in a number of studies seem to indicate a rational direction for research toward a cure for the disease. Ways to prevent Alzheimer's disease are also being explored.

Alzheimer's disease is believed to account for 50 percent or more of all cases of dementia—deterioration of intellectual capacity—among the elderly today. According to the National Institute of Neurological and Communicative Disorders and Stroke, an estimated 15 percent of people 65 years of age or older in the United States have varying degrees of dementia.



Al, a former city executive, suffers from Alzheimer's disease: ***he now attends weekly therapy sessions at a metropolitan hospital.***

Photo by Michael Anderson courtesy of Albert Einstein College of Medicine

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ALZHEIMER'S *continued*

The cause of the disease is not known. Two general theories, however, have been advanced. First, the disorder may be due to a general response of the brain to many injurious processes. Second, a single specific agent may cause the disease. Metal deposits, toxins, viruses, genetic factors, and immunologic abnormalities are suspected of being involved in the pathogenesis of Alzheimer's disease.

Dr. Alois Alzheimer, a German physician after whom the disease was named, first described the disorder in 1906 in the case history of a 51-year-old woman. Although usually associated with the elderly, onset of dementia in this disease may occur as early as 40 years of age. Some experts regard this dementia as being Alzheimer's disease, regardless of the age at which it occurs. Other experts regard the presenile and senile forms as different disorders; early-onset dementia is called Alzheimer's disease, and dementia with onset after age 65 is called senile dementia of the Alzheimer's type (SDAT).

Alzheimer's disease develops gradually and steadily, rather than suddenly. Clinical diagnosis of the disorder is based on the occurrence of a sequence of characteristic symptoms. Loss of memory for recent events is usually the first sign of the disorder, followed by impairment of judgment, comprehension, and other cognitive functions, which usually occurs within a year. Anxiety, depression, and other emotional disturbances often follow. Intellectual deterioration slowly worsens, and symptoms of neurological impairment such as difficulty in walking begin to appear. Finally the patient becomes bedridden with a deep dementia. Death usually occurs from infection or other complications about 5 to 8 years after onset.

Alzheimer's disease is diagnosed by a thorough physical, neurological, and psychiatric examination. An electroencephalogram (EEG) and brain scan usually supplement the clinical studies. "We've been impressed by the fact that the EEG is diffusely slow early in the course of the disease in patients who have the classical symptoms of Alzheimer's disease," says Dr. Marshal Folstein of Johns Hopkins University Medical School in Baltimore.

Dr. Folstein and his colleagues are currently investigating cases in which the disorder recurs in families. They are seeking clues to whether the disease might be due to transmission of a genetic trait or to common exposure to an environmental agent. There are no laboratory diagnostic tests as yet, but brain autopsy confirms clinical diagnosis.

He points out, however, that it is extremely difficult, if not impossible, to achieve early diagnosis with procedures currently available. "Clinical symptoms that identify Alzheimer's disease really take several years to develop to the point that permits confident diagnosis," Dr. Folstein says. "A longitudinal followup of the patient is required with clinical examinations to detect the sequence of classical symptoms of the disease and to rule out other neurological disorders, as well as cardiovascular disease. One must also look for a combination of Alzheimer's disease and some other disorder."

In making the diagnosis, diseases that mimic Alzheimer's disease must be ruled out. Multi-infarct dementia causes the greatest diagnostic difficulty. It appears to be responsible for 12 to 20 percent of all dementia in the elderly. Other causes such as brain tumors or lesions caused by minor strokes can be detected by computer-assisted tomography. Positron emission tomography (PET) can be used to search for abnormal physiological functions.

Tests of cognitive function are of paramount importance in the diagnosis of Alzheimer's disease. The signs and symptoms of Alzheimer's disease can be detected by the Mini-Mental Status Examination, which was devised by Dr. Folstein and is now used worldwide. The examination includes tests for recent memory, for attention, and for construction capacity, as well as for language skills, abstract reasoning power, and other cognitive functions.

The pathologic signs and symptoms detected are associated with senile plaques, masses of degenerating nerve material surrounding cores of an abnormal protein, and neurofibrillary tangles in the brain. In Alzheimer's disease these tangles occur in high density in the parietal area of the brain, Dr. Folstein says, as opposed to the small number that occur in normal subjects primarily in the temporal lobe.

Research designed to improve the diagnosis of Alzheimer's disease is being conducted along several different avenues. The search for a peripheral biological marker such as a blood test, a peripheral nerve abnormality, or a unique sign in one of the sensory organs is under way. Dr. Albert Heyman at Duke University Medical Center in Durham, North Carolina, is searching for a biological marker of Alzheimer's disease. Since he and his colleagues have evidence that the disease tends to run in families, they are looking primarily for a genetic marker.

The technique being used is known as linkage analysis for polymorphic gene markers in the blood. HLA typing, red blood cell typing, and serum enzyme studies are involved. Chromosome studies are also being carried out. One interesting chromosomal finding reported by Dr. Heyman is that, in comparison with sporadic cases and age-matched controls, "there is a small increase in frequency of premature centromere division of cells from female patients with Alzheimer's disease of the familial type."

Such aberrant chromosomes have been observed previously by other investigators and have been described as long acentric fragments. The frequency of this phenomenon is age-related and is not unique to Alzheimer's disease.

Family history studies of Alzheimer's disease patients by Dr. Heyman's group have yielded valuable information on familial occurrence of the disease. Approximately 20 percent of 68 patients studied at the Duke General Clinical Research Center (GCRC) over the past 3 years had either a sibling or a parent with Alzheimer's disease, Dr. Heyman reports.

Pedigree studies appear to indicate an increased frequency of Down syndrome among relatives of patients with Alzheimer's

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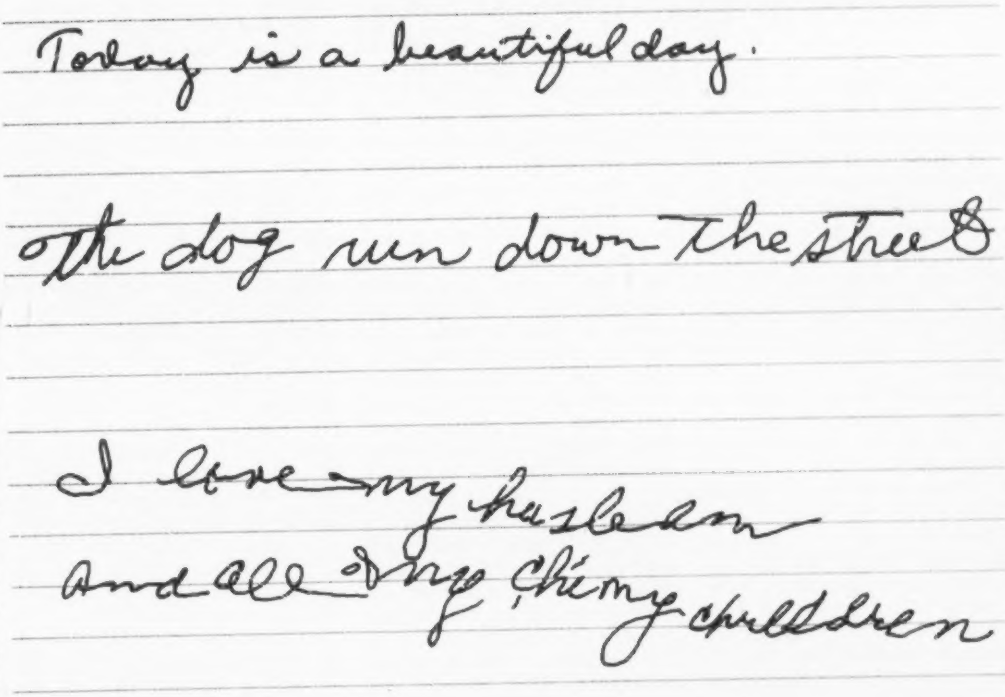
disease. The Duke study also indicates that Alzheimer's disease is more prevalent among women than among men.

Acetylcholine (ACh) is a substance involved in transmission of nerve impulses in highly evolved areas of the brain. The source of ACh and the mechanism of its loss in Alzheimer's disease were examined recently by Drs. Joseph T. Coyle and Donald Price and their colleagues at the Johns Hopkins University School of Medicine.

It is well established that in Alzheimer's disease there is a deficiency of ACh in areas of the brain responsible for the higher mental activities, suggesting that loss of the neurotransmitter might contribute to the impairment of memory, learning, judgment, and other mental activities that is characteristic of the disease.

For some time it has been thought that the loss of ACh in the cerebral cortex and hippocampus was due to death of neurons originating in these areas. However, during fundamental research on development of the cortex, Dr. Coyle and his colleagues found that chemical destruction of cortical nerve cells did not result in the loss of ACh in these areas of the brain. This means, Dr. Coyle says, that the cell bodies of the neurons that produce ACh in the cortex are located in some other part of the brain. The researchers discovered the location of these neurons in animals when they injected a chemical toxin into an area called the nucleus basalis (NB). Destruction of nerve cells in this area, deep in the brain, resulted in a dramatic loss of ACh in the cerebral cortex. Loss of ACh was also accompanied by a loss of choline acetyltransferase (CAT), an enzyme that catalyzes the synthesis of ACh in the nerve cell.

"When we produced a lesion in the brain of the rat, we reproduced the neurochemical pathology typical of Alzheimer's disease," says Dr. Coyle, "that is, ACh and CAT were markedly decreased in the cerebral



Today is a beautiful day.

othe dog run down the street

I love my hasleam
And all of my chimy children

Illustration courtesy of Dr Robert Katzman, Albert Einstein College of Medicine

Sentences written by a patient with Alzheimer's disease early after the disease was diagnosed (top) and later as it progressed: *silent evidence of mental deterioration.*

Senile plaque, large white area: *abnormal protein surrounded by bits of degenerated nerve fiber commonly found at autopsy in the brains of patients who had Alzheimer's disease.*

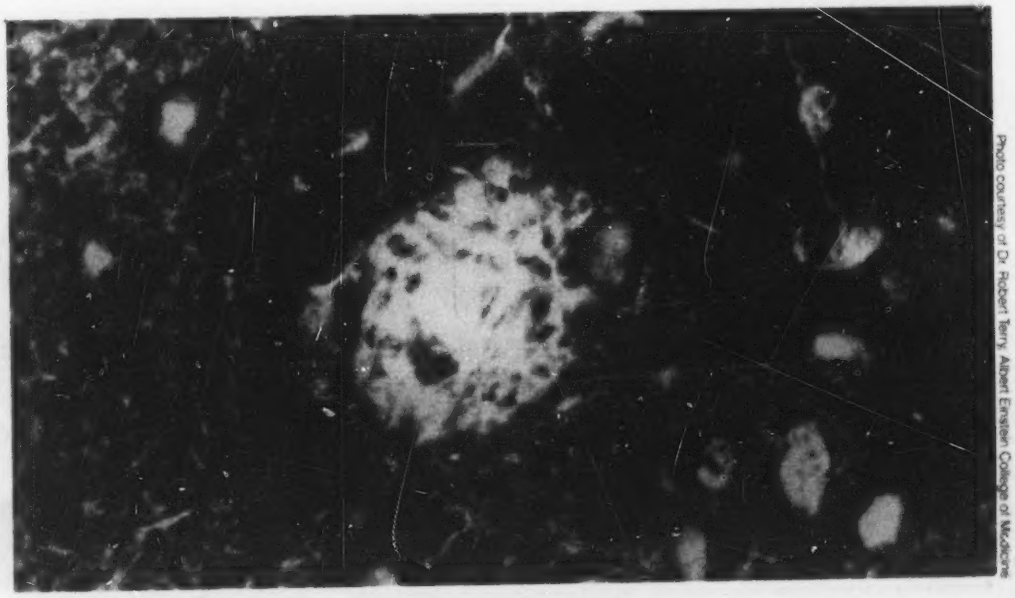


Photo courtesy of Dr Robert Terry, Albert Einstein College of Medicine

ALZHEIMER'S *continued*

cortex and hippocampus." When the connection between these findings and Alzheimer's disease was realized, Dr. Coyle teamed up with Dr. Price for an investigation of the role of the NB in Alzheimer's disease.

In their first autopsy, Dr. Price's team found that the loss of neurons in the NB of a 74-year-old Alzheimer's patient was 90 percent greater than the neuron loss in a normal, age-matched control. Moreover, some of the surviving nerve cells in the NB of the patient showed neurofibrillary tangles.

Five additional autopsies showed neuronal destruction in the NB of the same order of magnitude as in the initial case, Dr. Price says. These patients were in their late fifties or early sixties and died in end-stage dementia. Further studies suggested that senile plaques in the cerebral cortex, another common pathologic feature of Alzheimer's disease, resulted from destruction of neurons from the NB and not the neurons originating in the cortex as was previously supposed.

Dr. Coyle says, "We need to know a lot more about what the NB does and how its projections to the cerebral cortex are organized, because only after we understand this can we start considering rational approaches to try to treat the deficit that occurs."

Drugs that augment the activity of cerebral neurons using ACh as their neurotransmitter have been the type most widely evaluated in clinical trials with Alzheimer's disease patients. These trials have been based mainly on the discovery of a pronounced deficiency of ACh in higher brain centers, coupled with the knowledge that these cholinergic neurons are involved in memory and certain other cognitive functions. Researchers have hypothesized that increasing the amount of ACh, preventing its excessive breakdown, or prolonging its action at synapses might augment the action of cholinergic neurons that have not as yet been destroyed by the disease process. Studies in experimental animals and in normal human volunteers have shown that several of these drugs do enhance memory. Conversely, anticholinergic drugs have temporarily produced a memory deficit similar to that observed in patients with Alzheimer's disease.

Choline and lecithin, two ACh precursors, were the first substances tested in an effort to increase the amount of ACh in the cerebral cholinergic neurons of Alzheimer's disease patients. Choline is used by the body in the manufacture of ACh. Lecithin, a natural substance, is a dietary source of choline. It is found in a number of foods and is also a constituent of cell membranes.

Effects of dietary supplements of choline and lecithin in a number of clinical trials with Alzheimer's disease patients have been analyzed in a recent review of the literature by Dr. Suzanne Corkin of the Massachusetts Institute of Technology (MIT) GCRC in Cambridge. "Test results in general are not promising," says Dr. Corkin, "but many of the studies are inconclusive because of flaws in the experimental design or method."

In her review, Dr. Corkin points out that an adequate test of choline or lecithin therapy in Alzheimer's disease requires that: *"Patients be selected according to rigorous diagnostic criteria; a double-blind treatment protocol be used; drug or placebo be administered for at least 2 weeks and preferably longer; plasma choline levels be obtained as an index of compliance; the measures of behavioral tests be quantitative; the behavioral tests be at levels of difficulty that are suitable for elderly subjects. In addition, the data analyses should take account of practice and any deterioration in the patient's condition."*

A clinical trial in accordance with these requirements was conducted recently with 18 Alzheimer's disease patients at the MIT GCRC by Dr. Corkin in collaboration with Dr. John H. Growdon of the Tufts-New England Medical Center, Boston. The state of the disease in the subjects ranged from mild to severe.

Each patient was treated with 30 grams of a lecithin preparation containing 55 percent phosphatidylcholine or 20 grams of an 80 percent phosphatidylcholine preparation per day in three divided doses. The trial extended over a period of 18 consecutive weeks, including a mid-trial period of 2 weeks during which neither lecithin nor placebo was administered. Memory function was evaluated by four tests

Tangles of nerve tissue in the cortex of an Alzheimer's disease patient: ***twisted, delicate, thread-like neurofibrils in nerve cells.***

Photo courtesy of Dr. Robert Terry, Albert Einstein College of Medicine given periodically during the trial.

Dr. Corkin reported that lecithin did not produce statistically significant improvement in memory test performance. "Even isolated improvements in memory test scores or in home performance did not raise the mildest Alzheimer's patient to the level of a healthy elderly subject," he says. The researchers point out that erratic test performance and large variation in test scores could have masked minor beneficial effects.

Dr. Corkin believes that additional studies will be necessary to determine whether long-term lecithin administration can retard the progression of Alzheimer's disease.

A trial of lecithin treatment in Alzheimer's disease has also been conducted by Dr. Heyman. Eighteen patients, age 50 to 69 years, with mild-to-moderate dementia were enrolled in the study. Other causes of dementia were ruled out by case histories and by accepted standard diagnostic tests. According to a preliminary report by Dr. Heyman's team, results of the therapy were negative for all 18 patients.

The trial was a randomized, double-blind, double-crossover design in which each patient received three 8-week courses of lecithin or placebo. At the beginning of the trial and after each period of lecithin or placebo administration, cognitive function was tested with a battery of cognitive function tests including the Mini-Mental Status Examination.

Dr. Heyman says, "One of the major observations in this study was the decline in cognitive function that occurred during the 24-week period of the trial. Greater disorientation, further disturbance of immediate memory, and an increase in the severity of aphasia during this comparatively short period of time were indicated by the cognitive test results." He points out that rapid progression of deterioration is a serious obstacle to evaluation of therapeutic agents in Alzheimer's disease.

Dr. Heyman also emphasizes that "sufficiently large numbers of subjects with early, well-defined Alzheimer's disease must be selected and appropriate statistical analyses must be employed to determine the possible significance of cognitive or social changes observed during the clinical therapeutic trial."

Physostigmine, which reduces the rate at which the body breaks down ACh, was recently found to enhance memory in 10 Alzheimer's disease patients in a study conducted by Drs. Kenneth L. Davis and Richard C. Mohs at the Mount Sinai School of Medicine of the City University of New York and the Bronx Veterans Administration Medical Center, Bronx, New York. Patients were treated at the Mount Sinai GCRC in a randomized, double-blind manner with varying doses of drug or placebo. The trial was later repeated with the dosage of physostigmine that initially induced the best response. In both tests, all patients responded better to a recognition memory test when receiving physostigmine than when receiving placebo.

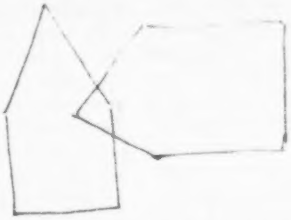
The patients, who ranged in age from 50 to 68 years, were selected for the study by careful and thorough diagnosis, using a battery of tests shown to identify those with a high probability of having Alzheimer's disease. Probability was based on diagnostic data that had been verified by autopsy. "Particular care was taken to rule out cases of multi-infarct dementia," Dr. Davis says.

The drug or a placebo consisting of ordinary salt solution was infused intravenously at a constant rate over a period of 30 minutes. Memory function was assessed by standard cognition tests—famous faces test, digit span task, and recognition memory test for either 24 words or 24 pictures. Dr. Davis explains that these tests measure the ability of subjects to store information in long-term memory. Testing began 5 minutes after the start of infusion of the drug or the placebo. It concluded 10 minutes after the infusion ended.

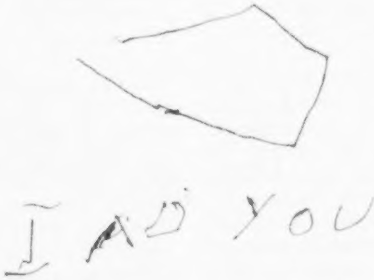
The most effective dose of physostigmine in the memory recognition test varied from patient to patient among the three doses tested: 0.125, 0.25, and 0.50 mg. Dr. Davis chose physo-

Testing Patients

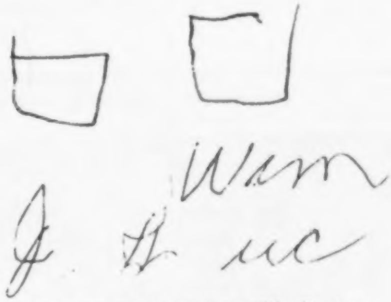
The following are samples of patient responses to two questions from Dr. Folstein's Mini-Mental Test. The patients were asked to re-draw the two intersecting pentagons shown below and to write a sentence. This demonstrates their ability to translate thoughts into motor skills.



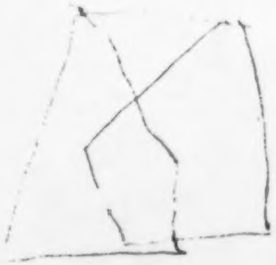
Patients asked to draw above design.



67-year-old male who has been ill for 3 years



73-year-old female who has been ill for 5 years



71-year-old male who has been ill for 6 years

ALZHEIMER'S *continued*

stigmine for the clinical trial because loss of memory is usually the first symptom of Alzheimer's disease and the drug has improved memory in both aged nonhuman primates and normal people.

Although physostigmine did not improve the patients' performance in all of the cognition tests, Dr. Davis says, "results of the study indicate that the acute augmentation of cholinergic activity in patients with Alzheimer's disease can partially reverse the memory deficit of that disorder and may provide an approach to the eventual therapy of the condition."

Combination therapy with physostigmine and lecithin has improved memory in Alzheimer's disease patients for up to 18 months in clinical trials conducted by Drs. Bruce Peters and Harvey Levin at the University of Texas Medical Center in Galveston. In the current study with six patients, Dr. Peters says, both drugs are given orally, and improvement seems to be about the same as in the earlier trial in which physostigmine was administered by subcutaneous injection along with oral lecithin.

Combination therapy with lecithin and physostigmine is a complementary approach to augmenting cholinergic activity in the higher brain centers. Lecithin provides additional choline for synthesis of the cholinergic neurotransmitter ACh, while physostigmine prevents its excessive breakdown by inhibiting the enzyme cholinesterase. "The six patients treated in the first trial showed improvement in memory as an aggregate group," says Dr. Peters. "Four showed clear improvement."

Each patient was given 2.4 grams of lecithin three times a day. The dosage of physostigmine varied from 0.5 milligram to 2 milligrams per day. The optimal amount of physostigmine must be determined by titration. "Too little isn't effective and too much can actually worsen memory functions," Dr. Peters says. Because the dosage for each patient was carefully adjusted, no adverse drug effects were encountered in the study.

Medication was discontinued occasionally to compare memory during and after treatment. The effect of treatment was determined by a modification of the selective reminding test, which consists of progressively learning a list of words, with selective reminding by the investigator of those words not remembered on the previous trial.

Dr. Peters cautions that, although the physostigmine-lecithin treatment definitely augments memory in certain Alzheimer's disease patients, "we do not wish to make any claim beyond that. We have no reason to think that this has any effect whatsoever on halting or modifying in any way the cause or natural consequences of Alzheimer's disease."

Best results with the physostigmine-lecithin treatment were obtained in patients with mild or moderate dementia. Patients in the moderately severe or severe categories do not appear to show any genuine improvement. Dr. Peters also points out that even some patients who initially showed improvement in memory stopped responding to therapy after a period of time—some after 18 months, some as early as a year after the start of treatment. "We believe that all of them will cease to respond eventually," he says. "This medication should be viewed as potentiating a function, but the function is progressively worsening despite the effects of the medication. At some point the medication ceases to overcome the progression."

Other drugs may be useful in reducing the effects of Alzheimer's disease. Arginine vasopressin, a neuropeptide synthesized by the endocrine hypothalamus, and a number of synthetic analogs of it have been found to enhance memory in experimental animals. One of the analogs, 1-desamino-8-D-arginine vasopressin (DDAVP), augmented learning and recall in Alzheimer's disease patients treated by a research team led by Dr. Herbert Weingartner at the National Institute of Mental Health in Bethesda, Maryland. Dr. Weingartner had previously discovered that DDAVP significantly improved learning and memory in normal volunteers.

In a preliminary trial, six early-stage dementia patients treated intranasally with 10 to 60 micrograms of DDAVP demonstrated improved memory and learning. "These patients could better access their previous knowledge," says Dr. Weingartner,

Nerve cells in the nucleus basalis of a normal brain (left) and a brain section from the corresponding area of a deceased patient who had Alzheimer's disease, showing a 90 percent loss of neurons, which are important to thought processes.

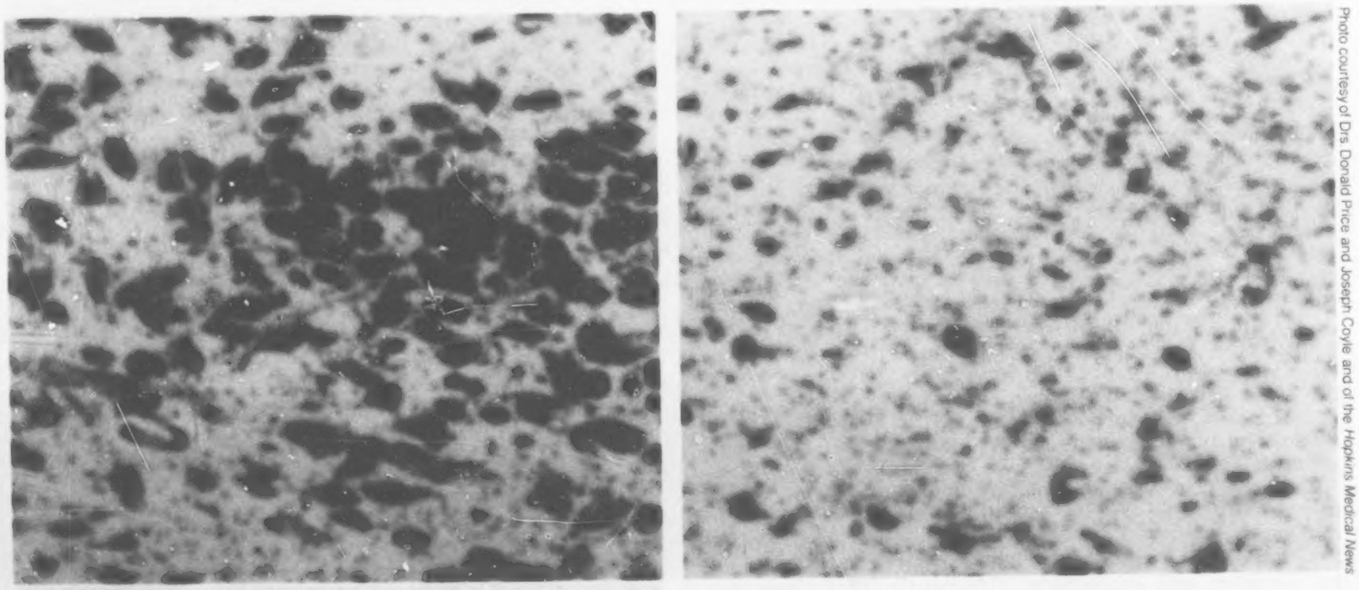


Photo courtesy of Drs. Donald Price and Joseph Coyle and of the *Hopkins Medical News* nd at least some of the subcts were able to learn someiat better."

The drug was administered veral times in a randomized, uble-blind, crossover study. ch patient also received a acebo at least three times.

Two types of tests were used evaluate the effects of DDAVP eatment on cognition. The first st measured access to preously learned knowledge; for ample, patients were asked to call words starting with the ame letter of the alphabet or ords of a single category such s animals. The second test easured learning and memory having the patient listen to fferent lists of 20 random words nd then recall as many as posble after a [illegible]-minute, activityled delay period.

Patients enrolled in the trial anged in age from 52 to 65 years nd were carefully screened for arly-stage, progressive idioathic dementia. Only patients hose history was consistent ith an Alzheimer's type of ementia were included, and all emonstrated clear and exensive memory impairments. ome patients are brought back om time to time for evaluaion of treatment in later tages of the dementia.

Dr. Weingartner points out hat the main objective of the tudies is to investigate the nature of cognitive impairment in atients with Alzheimer's disease r other similar disorders. Drugs sed in the trials serve as probes o learn how cognition fails in hese patients, says Dr. Weingartner. "I think this basic aspect of our research will ultimately be more valuable than some of the small therapeutic effects we've seen so far," he says. "It's important for people to appreciate he fact that we're not yet near achieving a promising therapeutic leverage on Alzheimer's disease."

One of the newest drugs advanced for consideration in the treatment of the disease is piracetam, a gamma-aminobutyric acid derivative. In a preliminary trial conducted by Dr. Eitan Friedman and his colleagues at the New York University School of Medicine, 3 of 10 patients with mild or moderate memory impairment improved both clinically and in memory function tests. The other patients showed "small, nonsignificant improvements in most cognitive functions," according to the researchers. Each patient was treated with 9 grams of choline and 4.8 grams of piracetam per day, in three equal doses, over a period of 7 days. A battery of objective cognitive function tests as well as behavioral rating tests were used to evaluate effects of the treatment. It has been suggested by some researchers that piracetam may act either by enhancing metabolism in the brain or by accelerating release of ACh in septohippocampal neurons to increase their firing frequency.

A new approach to the treatment of Alzheimer's disease has been designed by Dr. Leopold Liss of the Ohio State University School of Medicine GCRC in Columbus. Now being evaluated, it is based on Dr. Liss' hypothesis that aluminum plays a significant role in the cause of Alzheimer's disease and that its uptake and absorption can be controlled by precipitating the element in the gastrointestinal (GI) tract with sodium fluoride. It would then be excreted instead of being absorbed by the body.

Evidence that aluminum may be involved in the pathogenesis of Alzheimer's disease was obtained by Dr. Liss when aluminum he added to the diet of rabbits produced neurofibrillary tangles in the brain cells. Neurofibrillary tangles are characteristic of Alzheimer's disease. Furthermore, X-ray diffraction studies demonstrated that there was an elevation of aluminum in the nuclei of affected cells. Dr. Liss says that earlier studies have shown that there seems to be an etiologic connection between the deposition of aluminum and subsequent degeneration of neurons.

Since almost all human beings are exposed to aluminum from environmental sources—food, water, and dust—Dr. Liss hypothesizes that the human body contains a protective barrier, similar to the blood-brain barrier, against aluminum. He believes, however, that in some individuals this barrier is faulty, allowing aluminum to enter the brain and cause degeneration of neurons.

Dr. Liss points out that the underlying chemical principle for preventing the absorption of aluminum has been used for years in veterinary medicine to prevent fluoridosis in cattle. In this case, aluminum hydroxide is administered to combine with fluoride and thus prevent its absorption from the GI tract. Dr. Liss' treatment reverses the technique by administering sodium fluoride to prevent absorption of aluminum. "The effectiveness of our approach can be measured by simply determining the aluminum levels in the serum of our subjects," says Dr. Liss. "And measurements show that we are capable of effectively lowering the aluminum level in serum."

The first patients treated have been in early stages of Alzheimer's disease. "We are trying to help individuals who can still communicate with us, who still have sufficient attention span and can follow certain commands and participate in a number of tests," he says.

Although studies have not progressed very far, Dr. Liss says that in some instances the treatment seems to be working. In others, progressive degeneration to complete dementia has occurred despite the fluoride regimen.

Sodium fluoride is given orally three times daily at mealtime. The patients are carefully monitored for side effects such as fluoridosis, but no adverse conditions have been observed so far. Final results of the study will not be available for some time, but Dr. Liss believes that the preliminary results definitely justify further investigation of the hypothesis. ◼

Additional reading:

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2. Davis, K. L., and Mohs, R. C., Multiple dose intravenous physostigmine in Alzheimer's disease: Enhancement of memory processes. (Submitted for publication).
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4. Heyman, A., Logue, P., Wilkinson, W., Holloway, D., and Hurwitz, B., Lecithin therapy of Alzheimer's disease: A preliminary report. In *Alzheimer's Disease: A Review of Progress* (Aging, Volume 19) (Corkin, S., et al., eds.). New York, N.Y., Raven Press, 1982, pp. 373-378.
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Research into Alzheimer's disease described in this article was supported by the National Institute on Aging, the National Institute of Mental Health, the National Institute of Neurological and Communicative Disorders and Stroke, Ohio Department of Mental Health, Ohio State University College of Medicine Endowment Fund, Ford Foundation, Anna and Seymour Gitenstein Foundation, T. Rowe Price Foundation for the Study of Dementia, Unilever Research Laboratories, and Dr. Bruce H. Peters. The General Clinical Research Centers at Duke, Johns Hopkins, Mount Sinai, Ohio State, and the Massachusetts Institute of Technology are supported by the General Clinical Research Centers Program of the NIH Division of Research Resources. Collaborating with Drs. Joseph T. Coyle and Donald Price at Johns Hopkins were Drs. Arthur Clark, Mahlon DeLong, Michael Johnston, Michael McKinney, Robert Struble, and Peter Whitehouse.

Research Focus

Drug Delivery via Microscopic Biodegradable Beads

A new drug delivery system involving the use of microscopic biodegradable beads has been developed by researchers at the Ohio State University in Columbus. Drugs and other biologically active substances can be incorporated into the injectable beads for controlled release of the medication within the body.

The beads are prepared from serum albumin, a natural body protein. Consequently they are nontoxic and do not cause adverse immunologic reactions, according to Dr. Garfield Royer, professor of biochemistry and principal investigator. "The beads are small enough for hypodermic injection and are gradually degraded to harmless metabolic products by proteolytic enzymes in the tissues," says Dr. Royer.

The strong affinity of the serum albumin molecule for many drugs retards drug release until the bead undergoes biodegradation. Progesterone, morphine and its antagonist naloxone, dyes, peptides, proteins, steroids, and a number of anticancer drugs have been successfully packaged into the microbeads, which are only 1/2500 of an inch in diameter.

In rabbit studies with progesterone beads, serum hormone levels of 1 nanogram per milliliter were maintained for at least 20 days following a single subcutaneous or intramuscular injection. All beads injected intramuscularly had undergone absorption after 2 months. Repeated injections over a period of several months caused no adverse systemic effects or local inflammatory or necrotic reactions at the injection site. The new drug delivery system appears to eliminate much of the distress and tissue damage resulting from long-term treatment with drugs that must be administered frequently by injection.

Beads with an entrapped drug are formed by cross-linking polymerized serum albumin strands. Polymerization is initiated by addition of glutaraldehyde to a solution or a suspension in a buffer of serum albumin and a drug. The mixture is then poured into corn oil and petroleum ether and stirred at room temperature. Dr. Royer points out that cross-linking occurs mainly from the inside out. The surface of the bead consists largely of native

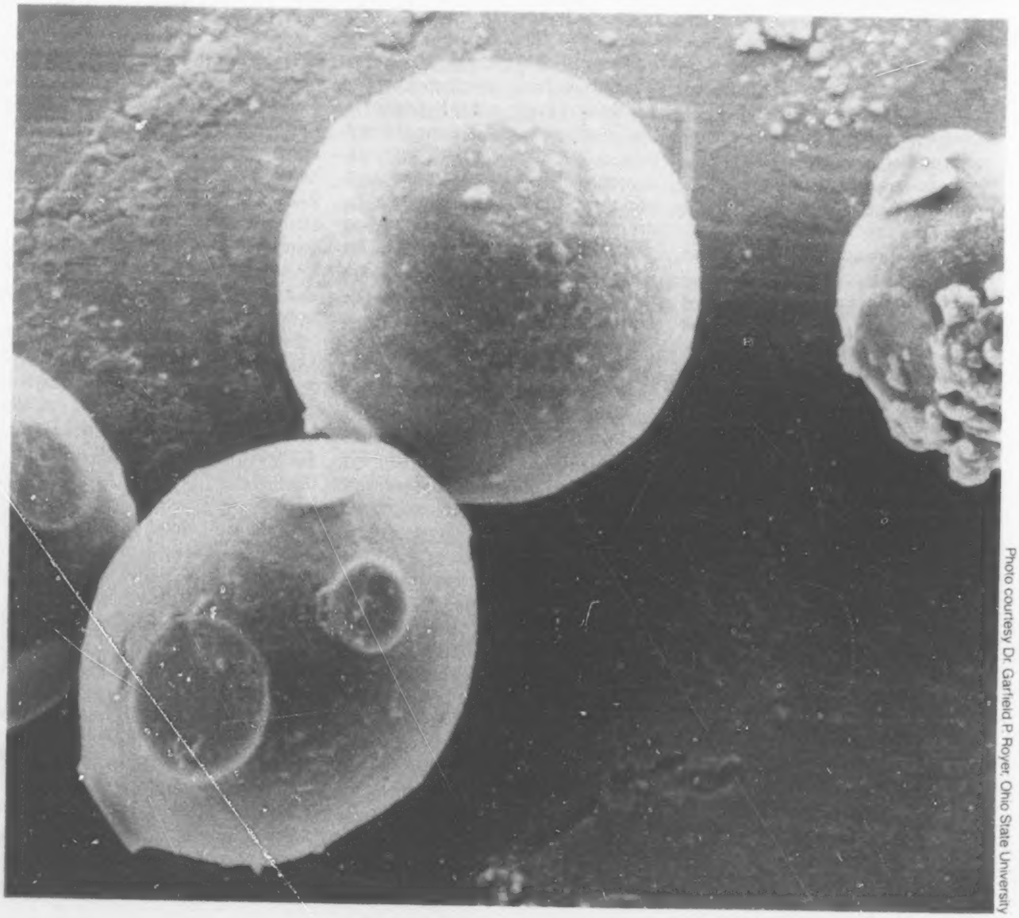


Photo courtesy Dr. Garfield P. Royer, Ohio State University

Serum albumin beads with entrapped drug: ***injectable beads release the drug gradually as tissue enzymes degrade the beads into harmless metabolic products.***

serum albumin. This is important, he emphasizes, so that the beads do not induce adverse immunologic responses when injected into the body.

Drugs are entrapped as the beads are formed. The drugs then crystallize during curing and drying. Drug content is controlled by varying the concentration of the drug in the reaction mixture. Beads with steroid concentrations as high as 30 percent have been prepared in this way by the researchers. "The beads retain the drug even after extensive washing to get rid of excess reagents," Dr. Royer says. Most biologically active molecules are stable under the conditions required to produce the beads.

Dr. Royer and his colleagues are currently attempting to prepare beads of just the right size to be entrapped in capillary beds. "We want to be able to localize the beads, to target them to specific places such as the capillary bed of a tumor," he says. Beads that adhere to capillary walls instead of circulating in the blood will permit prolonged diffusion of a drug to a tumor or an infection site.

The researchers are also studying the effects of a series of anticancer drug beads on tumor cells in tissue culture. Preliminary results, according to Dr. Royer, are encouraging. R

Additional reading:

Lee, T. K., Sokoloski, T. D., and Royer, G. P., Serum albumin beads: An injectable, biodegradable system for the sustained release of drugs. *Science* 213:233-235, 1981.

Research on the serum albumin bead drug delivery system is supported in part by the Biomedical Research Support Grant Program of the NIH Division of Research Resources. Dr. Timothy K. Lee, now with Lowell University, Massachusetts, and Dr. Theodore D. Sokoloski, professor of pharmacy at Ohio State University, were coinvestigators on the project.

PROPHET: A Network for Analyzing and Sharing Research Data

Cancer cells in a single tumor may grow at variable speeds. A unifying model that accounts for both fast and slow growth was developed with CELLSIM, a computer program that matches simulated cell growth to experimental data.

Digitalis, one of the most frequently prescribed heart drugs in the world, has widely different effects, depending on the form of the drug. The determining factor is the position of a single oxygen atom in the drug molecule, a discovery made with the computer program FITMOL, which allows chemists to correlate molecular structure with clinical activity.

CELLSIM and FITMOL are two of the programs available on PROPHET, a national computer network available to more than 700 scientists in government, academia, and industry. PROPHET was introduced 10 years ago by the Biotechnology Resources Program of the NIH Division of Research Resources (DRR) to support studies in drug development and toxic effects of chemicals. Today the system is used in virtually all areas of biomedicine.

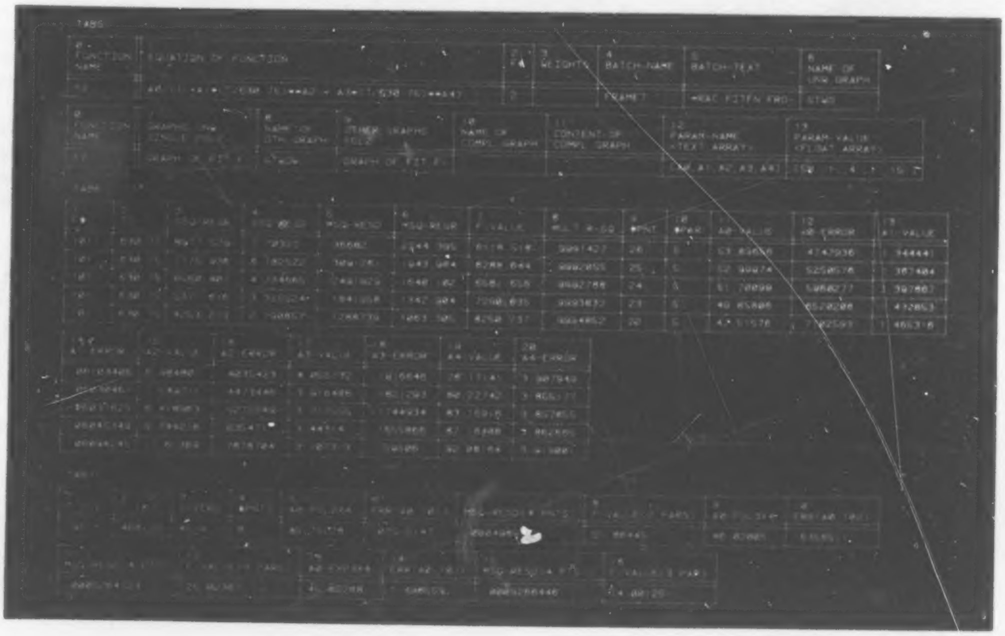
"PROPHET stimulates productivity and creativity by giving investigators the opportunity to obtain the maximum amount of information from their data and to be intimately involved in the analysis," says Dr. Jack Hahn, DRR project officer of the PROPHET system.

According to Dr. Hahn, data analysis by investigators who conceived and personally conducted the research provides major advantages. "Knowing how the work was done and being familiar with the data, investigators may be able to identify subtle relationships that might be missed by someone not as familiar with the work," he says.

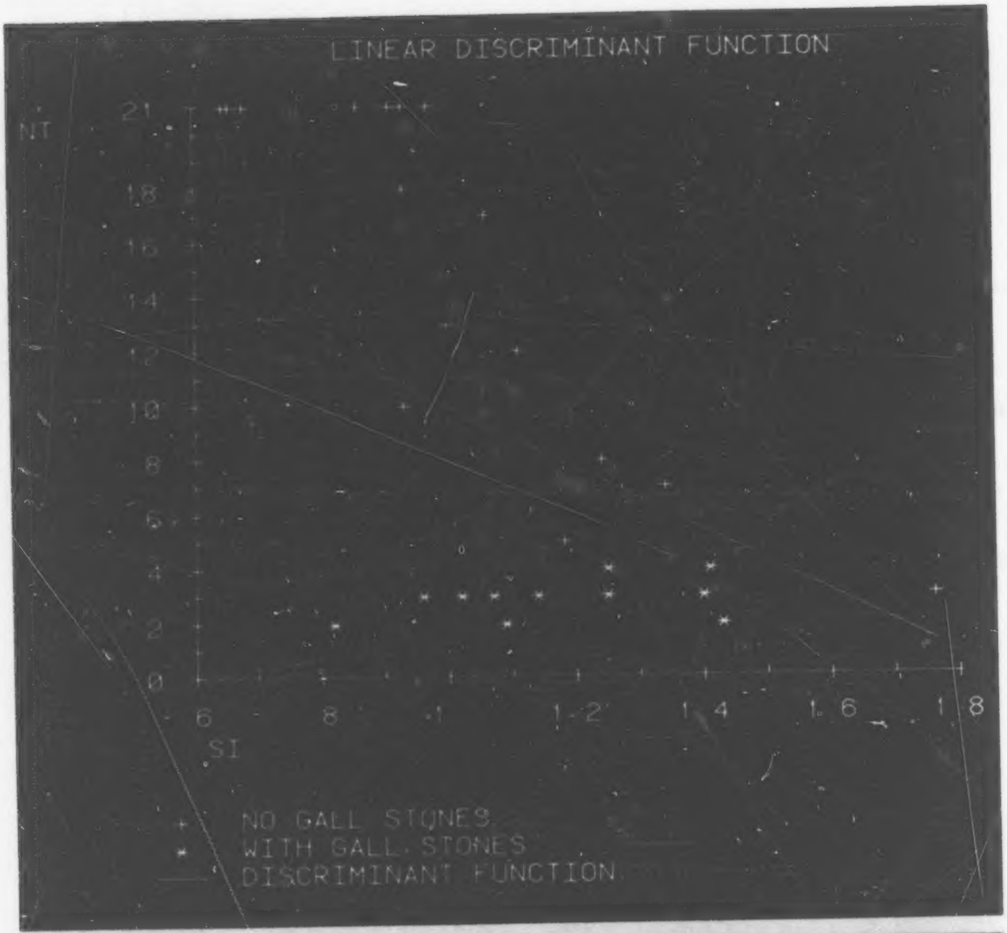
In one sense, PROPHET is an automated laboratory notebook. It can be used to store, manipulate, and graphically display experimental data. In another sense, the system is a tutor and conduit of information, guiding the researcher through the use of a wide variety of tools for data analysis and providing the vehicle for sharing data with collaborators around the nation.

"PROPHET is much more than a computer; it is a social network composed of researchers who rely on each other for knowledge, assistance, and new techniques of analysis," Dr. Hahn says. PROPHET also serves as a research information channel between research institutions and particularly among the community of DRR resources; for example, it currently links the Oregon Regional Primate Research Center, the Beth Israel Hospital General Clinical Research Center, and five schools in the Minority Biomedical Research Support Program.

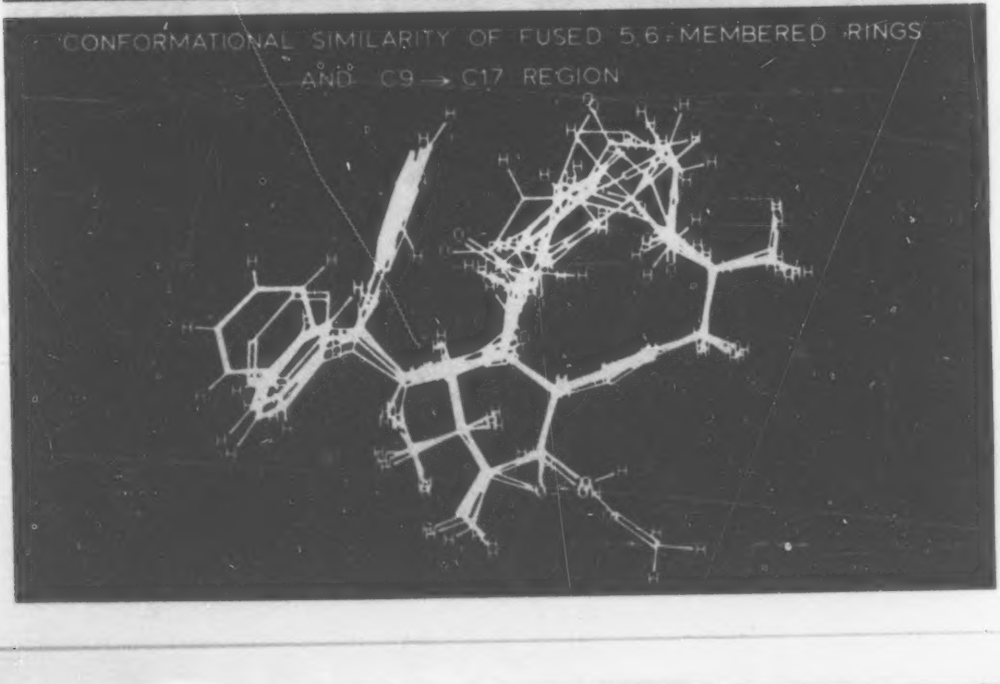
In addition to strengthening the national research effort, PROPHET reduces the cost of research. "The system shifts the



PROPHET table of experimental data: *automating the laboratory notebook.*



PROPHET graph of results from clinical study of gallstones: *plotting data with the command MAKE GRAPH.*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PROPHET: *a sketch pad for the biochemist.*

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nler, director of the DRR Bio-
nnology Resources Program,
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k.

Vhen using PROPHET, the in-
stigator typically employs
nmands that are similar to
nmon English phrases. With
command MAKE TABLE,
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ular format of columns and
vs. Data are plotted with the
nmand MAKE GRAPH. Func-
nal relationships are estab-
ned with the commands FIT
NE or FIT FUNCTION. And if
project involves more than
e set of data, these sets can be
tistically analyzed with the
mmand COMPARE.

To the biochemist, PROPHET
a sketch pad on which outlines
molecules can be drawn with
digitizing pen, displayed in two
three dimensions, and scaled,
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of their structural relation to
ch other.

Help in using PROPHET's
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oup based in Cambridge, Mas-
chusetts, at Bolt, Beranek, and
ewman, Inc. (BBN), which con-
cts the project under contract
th DRR. "Help in using the
ftware or in solving PROPHET-
lated problems is only a phone call away," says Dr. Jim Wood, assistant project director at BBN.

Members of the user assistance group typically visit each of the 40 PROPHET sites five times a year. Feedback from network users is also obtained through yearly colloquiums, at which individual researchers and special interest groups, formed by investigators with similar research interests, discuss how PROPHET might evolve to meet their emerging needs. The end product of phone consultations, site visits, and the annual colloquium is a system that is highly responsive to the needs of users, says Dr. Hahn.

Because PROPHET is simple to use, it can be applied by researchers with no knowledge of computer science. "Any given area of science is so complex and demanding that few scientists can be well informed in a specialty *and* be an expert in computer science as well," says Dr. Hahn. "PROPHET contains the necessary tools and capabilities to let the investigator concentrate on the research problems at hand."

The system, however, does not exclude researchers with a talent for programming. New programs, written in PL/PROPHET, a powerful computing language, can be developed to meet special needs. And once these programs are on the PROPHET system, they are often shared with other members of the network.

Programs developed initially for determining the sequence of amino acids, for example, have been broadly applied by various researchers in DNA and RNA sequence handling, allowing the analysis of secondary structure and sequence alignment. These and other programs, available through telephone links to a central computer in Cambridge, service a widely dispersed community of scientists and provide the basis for joint research projects.

Using PROPHET, pharmacologists in Oregon who have synthesized a drug can collaborate with researchers in New York to examine the drug's molecular structure. Both groups can then work with clinical investigators in Minnesota to evaluate the drug's effects. This flexibility in access to data regardless of location may be further exploited by researchers who want to move to a different laboratory or take sabbatical leave, allowing them to move across the country and still maintain contact with a research team left behind. By organizing and presenting data more effectively and by providing a link to research projects going on across the nation, PROPHET helps academic institutions to obtain grants and high-quality personnel, according to Dr. Hahn.

The use of PROPHET is subsidized by NIH for academic scientists, who comprise the majority of the network users. About 30 of the 40 user sites are based at colleges and universities. But a number of Federal agencies and commercial firms are also included in the community.

The National Institute of Child Health and Human Development applies the PROPHET system in its study of contraceptives. The National Institute of Environmental Health Sciences uses PROPHET in its toxicology program. The National Eye Institute uses PROPHET in its Laboratory of Vision Research. Four other NIH institutes jointly sponsor the application of PROPHET in the tabulation and analysis of amino acid sequences in immunoglobulin chains. PROPHET helps Food and Drug Administration scientists study the kinetics of drugs and may soon be used as an aid in reducing the time and cost of reviewing new drug applications made by pharmaceutical companies. Three commercial organizations also use PROPHET.

"All segments of the PROPHET community—academia, government, and industry—contribute to the system by suggesting new computer techniques, by sharing expertise, and by writing and sharing programs," says Dr. Hahn. "It is particularly encouraging that commercial users follow this philosophy, demonstrating a spirit of community rather than competition."

As the network expands to include more investigators, cost-effectiveness increases. System capabilities also expand as the system draws from the influx of new expertise, knowledge, and experience. Says Dr. Hahn: "PROPHET enhances the sophistication of scientists and in doing so the system is beginning to have a significant impact on creativity and productivity in national biomedical research."

Research Resources Reporter
June 1982

Information Exchange

Research Highlights

A significant segment of the medical community believes that computer-based consultants will ultimately help clinicians, but acceptance of these computer systems depends on their performance and their ability to interact with the physician, according to a survey conducted by Stanford University scientists.

The survey included opinions drawn from 146 physicians representing a variety of medical fields, including internal medicine, pediatrics, surgery, radiology, and pathology. Respondents stressed the importance of the capability of computerized consultants to explain their advice and to understand and update their knowledge automatically. Respondents demanded neither perfect diagnosis nor perfect treatment planning. They did not recommend that the amount of technical knowledge required of a physician be reduced, regardless of how well a consultation system performs, and they would not accept a consultation system as a standard for medical practice.

The primary objective of the survey, conducted by Dr. Edward H. Shortliffe and Mr. Randy L. Teach of Stanford University, was to measure physicians' attitudes that are important to the successful introduction of computerized consultants into clinical practice. "The survey documented characteristics that physicians believe must be included in systems if these systems are to be practical," says Dr. Shortliffe, a computer scientist and systems developer. "It also examined what physicians are afraid will occur when computers begin to play a more prominent role in clinical practice."

Dr. Shortliffe and Mr. Teach found that a key concern is that these systems be designed to assist rather than direct physicians in making decisions. Citing the success and failure of several medical technologies, Dr. Shortliffe explains that the distinction between a *clinical aid* and a *replacement* is crucial.

Computed tomography (CT), for example, has been widely accepted as a useful tool in clinical management, providing accurate information quickly to the physician. But automated history-taking systems have been largely rejected by the medical community, despite their accuracy and reliability. "We suspect that one reason physicians have rejected history-taking systems is that they are perceived as a threat to the traditionally personal role of the physician," says Dr. Shortliffe. According to the survey, computer systems should ultimately perform some duties now done by the physician, if the result is more efficient and less costly health care; but they must not infringe on the physician's role as chief architect of the patient's management.

The survey indicates that physicians prefer systems with humanlike qualities, particularly the ability to explain recommendations. "Respondents in our study accept that no one is infallible, and they do not expect a computer system to be any better; but they want the system to explain what it's doing so they can decide when it's making mistakes," says Dr. Shortliffe.

Researchers in artificial intelligence (AI) are trying to develop systems that mimic the thinking processes of human experts. This ability is essential if computers are to explain their advice in terms acceptable to the clinician, and it may also be an essential quality of reliable systems. Says Dr. Shortliffe: "Many of us believed in the 1970's that it didn't matter how the computer solved the problem as long as it was able to explain it in terms that the physician can understand. Evidence increasingly suggests, however, that optimal decision-making by machines requires a duplication of the ways by which expert physicians make decisions."

One of the most advanced AI consultation systems intended for medical application relies heavily on this human mimicry. A system known as Caduceus, being developed at the University of Pittsburgh, can diagnose unrelated diseases. Before rendering its opinion, Caduceus sifts through medical data provided by the physician, considers several diagnoses simultaneously, and asks for additional information when necessary.

Caduceus, which is scheduled to undergo clinical tests in Pittsburgh later this year, is one of more than a dozen AI systems being developed by SUMEX-AIM (Stanford University Medical Experimental Computer-Artificial Intelligence in Medicine). SUMEX-AIM is a nationwide network of AI researchers striving to build computerized consultants for application in biomedicine. Most systems are still in early stages of development, but some have entered their respective fields. PUFF is being used routinely at the Pacific Medical Center in San Francisco to evaluate lung performance in patients undergoing pulmonary tests. Developed by researchers at Stanford and the Pacific Medical Center, PUFF interprets data and produces typed analyses. These reports are seldom challenged by attending physicians.

"It's important to note that physicians are not asked to go to a PUFF terminal and request a consultation," says Dr. Shortliffe. "The use of PUFF differs from the use of most other AI systems, which require direct interaction with the physician."

To be accepted by physicians, Dr. Shortliffe emphasizes that simplicity of interaction between physician and machine is crucial, while updating the system's knowledge base should not require the physician's time. Ideally, the systems should parallel the reasoning of a human expert.

Although these abilities cannot be entirely developed with current technology, the future is promising, says Dr. Shortliffe. Researchers are now programming computers with reasoning processes that mimic those of the human mind, processes that can be explained by the computer in medical terms. These systems are being designed for operation by users with minimal knowledge of computer science. And text reading programs are being studied as ways to update the computer's knowledge base.

"We cannot predict exactly when systems with all these abilities will be available," says Dr. Shortliffe, "but in defining what is necessary for AI systems to be accepted, we have established specific goals—and these goals can be met."

Additional reading:
Teach, R. L., and Shortliffe, E. H., An analysis of physician attitudes regarding computer-based clinical consultation systems. *Computers and Biomedical Research* 14:542-558, 1981.

The Stanford University survey of physicians' attitudes toward computerized consultation systems was supported by the Kaiser Family Foundation, the National Library of Medicine, and the Biotechnology Resources Program (BRP) of the NIH Division of Research Resources. SUMEX-AIM is funded through the BRP.

Research Service

High Voltage Electron Microscopy Resource

The High Voltage Electron Microscopy (HVEM) Resource at the New York State Department of Health in Albany is available to investigators studying cell biology, particularly the interrelationships of cell structures in thick-section and whole-cell mounts. The instrument allows tomographic analysis by computerized reconstruction of multiple-tilt photographs and three-dimensional imaging of cellular structure by stereoscopic viewing.

Cell structures such as tubules, filaments, nuclear pores, and ribosomes can be studied at a maximum resolution of 4 angstroms. The HVEM provides images at 1.2 megavolts, compared to conventional transmission electron microscopes, which have capabilities of only 0.1 megavolt. With the HVEM, sections 60 times thicker and greater in volume than those used with conventional microscopes can be studied.

The resource, which is supported by the Biotechnology Resources Program (BRP) of the NIH Division of Research Resources, is directed by Dr. Donald F. Parsons, who is using the instrument to study human squamous cell carcinomas. He has found that tonofilaments support the formation of cage-like structures around the nucleus. Studies of such interrelationships cannot be accomplished by using conventional microscopes.

Instruments available at the resource include an AEI-Kratos HVEM, model EM-7 Mk2. An image-processing unit, a double-tilting stage, stereo viewers, and a dark-field imaging unit are available. The image-processing unit contains a digitizing densitometer.

The HVEM at Albany currently is one of the few instruments, if not the only such instrument, in the world with a fully functional image-processing unit devoted to biomedical research. Most instruments of similar sophistication are used mainly for the study of metals, because of their exceptional penetration power. There are three other HVEM's in the United States that are dedicated to biomedical science but lack image-processing units. These are at the University of Colorado in Boulder, the University of Wisconsin in Madison, which are both supported by BRP, and the University of California in Berkeley.

The Albany instrument is available for use 49 hours per week at no charge. Dr. Parsons and his staff train visiting scientists who are not familiar with operating techniques. Investigators who want to discuss proposed research should contact:

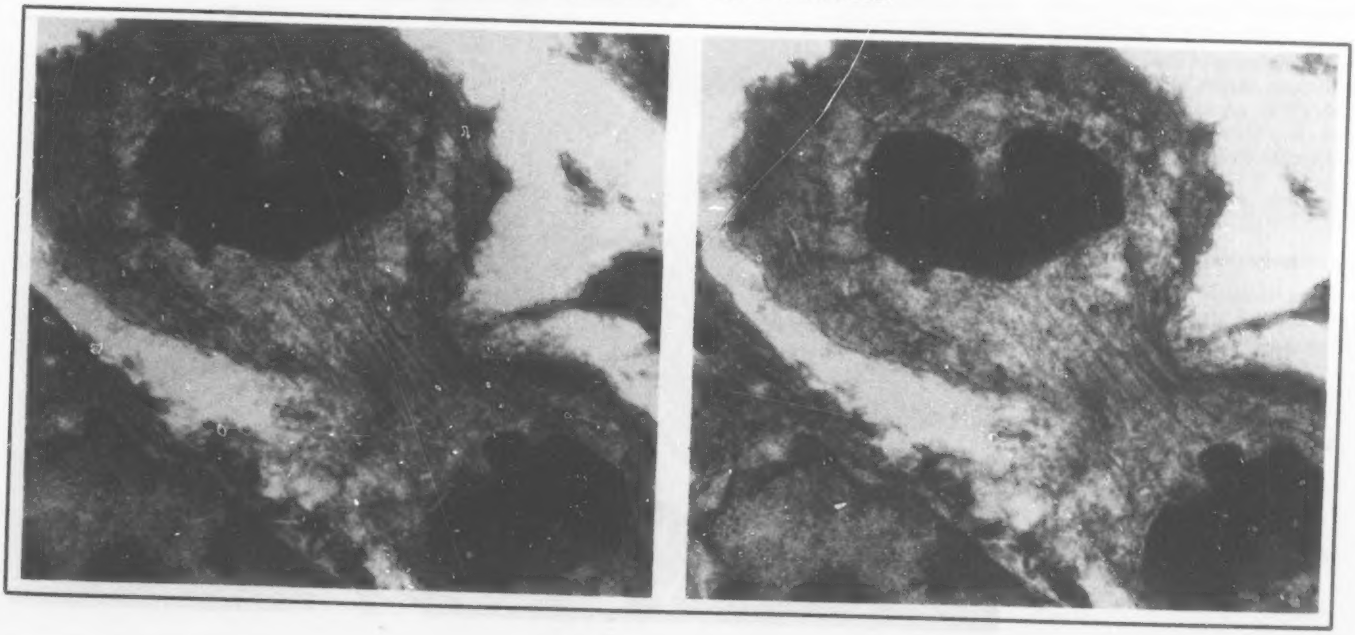
Dr. Donald F. Parsons
Division of Laboratories and
Research
New York State Department of
Health
Empire State Plaza
Albany, New York 12201
Telephone (518) 474-7047

Film Describes Computer Tracing of Nerves

A 15-minute film describing a computer technique that allows researchers to construct three-dimensional models of nerve systems is available on loan to interested scientists, researchers, and instructors.

Produced at Columbia University in New York, the film entitled *CARTOS: Visualizing Nerves in Three Dimensions* illustrates with color computer graphics the different types of research possible with the use of computer-aided reconstruction and tracing of serial sections (CARTOS).

"Whenever you are dealing with complicated three-dimensional structures, whether they are cells, or groups of cells, or nerve tracts running from one place to another, you have to be able to put that information into a three-dimensional framework in order to make it understandable," says Dr. Cyrus Levinthal, inventor of CARTOS and principal investigator of the Biological Computer Facility for Image Processing and Displays



Dividing cancer cell from the tonsil photographed in stereo using a double-tilt stage: ***stereo viewing reveals tonofilaments that form cage-like structures around the nucleus.***

at Columbia. "That is the greatest value of CARTOS. In simplest terms, it is a three-dimensional notebook."

Traditionally, scientists have visualized nerve structures by assembling micrographs or by tediously constructing models from plastic or wire. CARTOS simplifies this process by storing all the individual micrographs in a computer memory so that the entire image can be viewed either as a whole or as parts.

Large areas of simple nervous systems such as those in zebra fish, daphnia, or leeches can now be modeled in minute detail. Larger structures such as the human brain can also be modeled, although not as completely.

"CARTOS is really the only technique that lets you see nervous systems at the most detailed level, where you can see all the connections of all the cells," says Dr. Irwin Sobel, a member of the group at Columbia's Biological Computer Facility for Image Processing and Display. "The simple act of viewing the reconstructed objects on a television screen and manipulating them in three dimensions can yield valuable scientific insights."

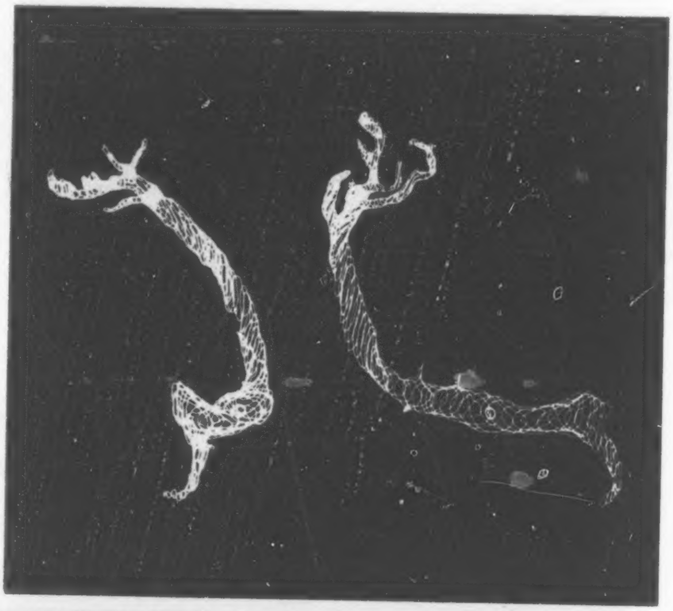
Since Dr. Levinthal and his associates constructed the first CARTOS system at the facility, other researchers have built similar systems, each for a slightly different purpose. Most applications have been in biomedicine, but some include applications to various problems in the geological sciences.

To enable other investigators to develop systems on their own, Dr. Levinthal, Mr. Noel Kropf, and their colleagues have built a portable CARTOS unit, which can be lent to investigators around the country. In addition, the staff at the facility helps to develop or refine systems for outside investigators and gives advice on solving research problems. The loaner unit serves those who cannot leave their own research facilities for extended periods of time.

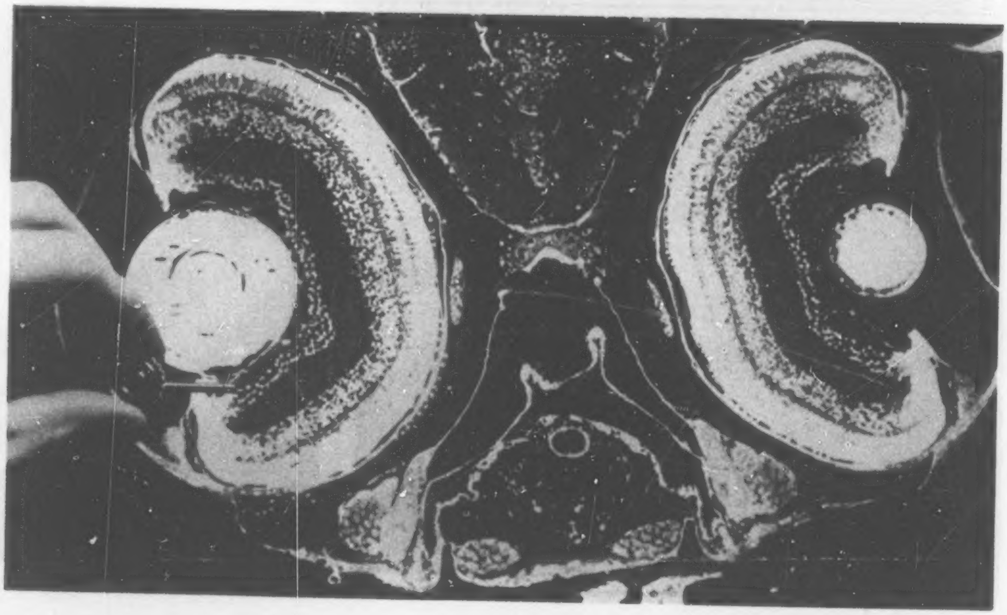
Although the loaner CARTOS has fewer capabilities than the parent system at Columbia, it can be used to trace data into the computer directly from micrographs. Once tracings have been recorded, the model can be viewed on the display unit built into the portable device, or the data can be transferred to the Columbia facility, where sophisticated data analysis can be done and display equipment can provide better resolution and dynamic three-dimensional rotations.

Steps required to construct computer models of nerves are explained and demonstrated in the film. The basic procedure involves dissecting the animal, fixing and staining the tissue, and embedding it in paraffin or plastic. The nerve tissue is then sequentially sliced. Micrographs of tissue sections are then traced into a computer system, which assembles the many slices into a three-dimensional model. The system

Cells from the zebra fish brain as imaged by CARTOS: *computer reconstruction of micrographs simplifies an otherwise tedious and time-consuming task.*



Manual tracing of the forebrain and optic nerves of the newborn zebra fish: *an early step in constructing a three-dimensional computer model of neuronal tissue.*



can selectively picture a nerve midsection, an entire nerve, or even all the major nerves in a structure as complex as a mammalian brain.

The film is accompanied by an illustrated booklet that briefly describes CARTOS. Loan of the film and bulk copies of the booklet are free of charge. For further information, write to: Research Resources Information Center, 1776 East Jefferson Street, Rockville, Maryland 20852.

Development and operation of the CARTOS systems at Columbia University are supported by the Biotechnology Resources Program (BRP) of the NIH Division of Research Resources. The Biological Computer Facility for Image Processing and Displays is supported by the BRP.

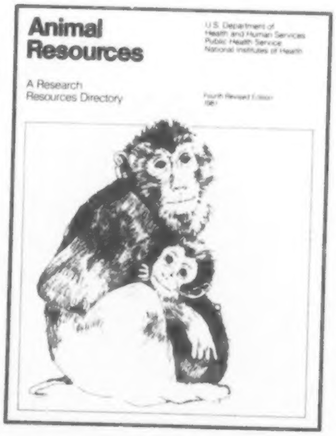
Revised Animal Resources Directory Available

The revised 1981 directory of the Animal Resources Program (ARP) has been published and is now available. Titled *Animal Resources, A Research Resources Directory*, the booklet is a guide for scientists seeking sources of assistance and collaboration in health research involving nonhuman primates and other laboratory animals.

The 50-page directory identifies animal diagnostic laboratories, animal information projects, animal reference centers, special colony and model study centers, and the major primate research centers currently supported by the Animal Resources Program of the NIH Division of Research Resources. Services and facilities available at the resources include modern analytical laboratory equipment, surgery, X-ray and clinical pathology units, improved animal cages and holding facilities, material and techniques for diagnosis of animal diseases, reference reagents and antisera, animal breeding stations, and selected invertebrate and vertebrate species, including specific genetic strains.

The directory includes information on resource location, research emphasis or application, the principal investigator, and his or her address and telephone number. A geographic index lists the resources by state and by address in alphabetical order.

A single free copy of the directory may be obtained by writing to the Research Resources Information Center, 1776 East Jefferson Street, Rockville, Maryland 20852, or by request from the Office of Science and Health Reports, Division of Research Resources, National Institutes of Health, Bethesda, Maryland 20205.



Animal Resources

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

A Research Resources Directory

Fourth Revised Edition
1981

Note on Pseudohermaphroditism

In the May 1982 issue of the *Research Resources Reporter*, the article entitled "Pseudohermaphrodites: Models of the Developing Male," two countries, Brazil and Mexico, were not listed among those having reported cases of male pseudohermaphroditism, a condition that masks the true sex of children until puberty causes male genitalia to become recognizable.

The article incorrectly stated that 17-beta-hydroxysteroid dehydrogenase deficiency induces the condition by preventing the conversion of testosterone to dihydrotestosterone. In actuality, the deficiency induces male pseudohermaphroditism by preventing the conversion of testosterone to androsterone, according to Dr. Ralph E. Peterson, director of the endocrinology division at Cornell University.

During puberty, testosterone production increases tenfold to twentyfold in subjects with 5-alpha-reductase deficiency, causing male genitalia to develop.

Dr. Peterson, who was inadvertently identified with a picture of his colleague, Dr. Teofilo Gautier, emphasizes that the disease is rare. Only six cases have been reported in the United States, although dozens have been reported in other countries. Most of the children in the U.S. were castrated before or during puberty. Castration and lifelong female hormone therapy might abort the physical change from female to male, but there is no guarantee that sexual imprinting on the brain by male hormones will be reversed.

Although outweighed by the psychological trauma, the condition offers certain physiological advantages. A receding hairline, hair loss, or prostate enlargement have not been reported in subjects.

A fast, accurate method of diagnosing the condition due to 5-alpha-reductase deficiency has been developed. The test requires the measurement of several urinary steroids including etiocholanolone and androsterone. The ratio of 5-beta tetrahydrocortisol to 5-alpha tetrahydrocortisol is markedly increased at 20 to 50. The normal ratio is 4 to 1.

For additional reading on male pseudohermaphroditism, see:

1. Imperato-McGinley, J., Guerrero, L., Gautier, T., and Peterson, R.E.: Steroid 5-alpha-reductase deficiency in man: an inherited form of male pseudohermaphroditism. *Science* 186:1213, 1974.
2. Peterson, R. E., Imperato-McGinley, J., Gautier, T., and Sturla, E.: Male pseudohermaphroditism due to steroid 5-alpha-reductase deficiency. *American Journal of Medicine* 62:170, 1977.

Meetings

June 14-17, 1982

Title: *International Workshop on Blood-Gas Sensor and Measurement Technology*
Location: *Columbia, Maryland*
Contact: *Dr. Michael R. Neuman*
Biomedical Electronics Resource
Electronics Design Center
Case Western Reserve University
Cleveland, Ohio 44106
Telephone (216) 368-2926

June 14-18, 1982

Title: *6th International Symposium on Atherosclerosis*
Location: *West Berlin, Germany*
Contact: *Professor Gunter Schlierf*
c/o Medizinische Universitatsklinik
Bergheimer Strasse 58
D6900 Heidelberg,
West Germany

June 15-24, 1982

Title: *Regulatory Elements of the Immune System*
Location: *Durham, North Carolina*
Contact: *Dr. David W. Scott*
Box 3010
Division of Immunology
Duke University Medical Center
Durham, North Carolina 27710
Telephone (919) 684-3388

July 7-10, 1982

Title: *26th Annual Meeting of the Society for Research in Hydrocephalus and Spina Bifida*
Location: *Boston, Massachusetts*
Contact: *Mr. D. G. Young*
Royal Hospital for Sick Children
Glasgow G3 8SJ, Scotland

July 14-16, 1982

Title: *Ions, Cell Proliferation, and Cancer*
Location: *Lake Placid, New York*
Contact: *Dr. W. L. McKeehan*
W. Alton Jones Cell Science Center

Old Barn Road
Lake Placid, New York 12946
Telephone (518) 523-2427

July 15-17, 1982

Title: *5th National Buffalo Conference on Pediatric/Adult Allergy and Clinical Immunology*
Location: *Toronto, Ontario, Canada*
Contact: *Rayna Dutton*
Continuing Medical Education
Children's Hospital
219 Bryant Street
Buffalo, New York 14222
Telephone (716) 878-7630

July 22-24, 1982

Title: *14th Conference on National Advances in Pediatrics*
Location: *Atlantic City, New Jersey*
Contact: *Rayna Dutton*
Continuing Medical Education
Children's Hospital
219 Bryant Street
Buffalo, New York 14222
Telephone (716) 878-7630

July 26-30, 1982

Title: *3rd Meeting of the World Federation for Ultrasound in Medicine and Biology*
Location: *Brighton, England*
Contact: *WFUMB 82*
Conference Office
4 'L' Portman Mansions
Chiltern Street
London WIM ILF, England

July 27-30, 1982

Title: *28th Annual Meeting of the International Committee on Thrombosis and Haemostasis*
Location: *Bergamo, Italy*
Contact: *Dr. H. R. Roberts*
University of North Carolina
Department of Pathology
Preclinical Education
Building 228-H
Chapel Hill, North Carolina 27514
Telephone (919) 966-1360

August 8-13, 1982

Title: *9th Congress of the International Primatological Society and Annual Meeting of the American Society of Primatologists*
Location: *Atlanta, Georgia*
Contact: *Dr. Frederick A. King*
Director
Yerkes Regional Primate Research Center
Emory University
Atlanta, Georgia 30322
Telephone (404) 329-7707

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This Issue:

Microsphere Enzyme Delivery
Cell Changes and Bladder Cancer
PET Imaging Agent
Histocompatibility

by
Donald W. McKinstry, Ph.D.

Some Animal Models of Diabetes Mellitus

Diabetes mellitus is one of the least understood and most prevalent diseases of man, affecting an estimated 10 million Americans, according to the National Diabetes Data Group. To develop a better understanding of the disease, animal models that mimic human symptoms have been developed. Some models have been studied for nearly 100 years, according to the Task Force on Animals Appropriate for Studying Diabetes Mellitus and Its Complications. The task force, convened by the NIH Diabetes Coordinating Committee, states in its report that animal research toward the development of insulin therapy provided the basis for significantly extending the lives of human diabetics. Now that diabetics are living longer, the need for animal research into the etiology and chronic complications of diabetes mellitus has grown, the task force asserts.

One model that is providing needed information occurs in the Celebes ape, also known as *Macaca nigra*. Incidence of

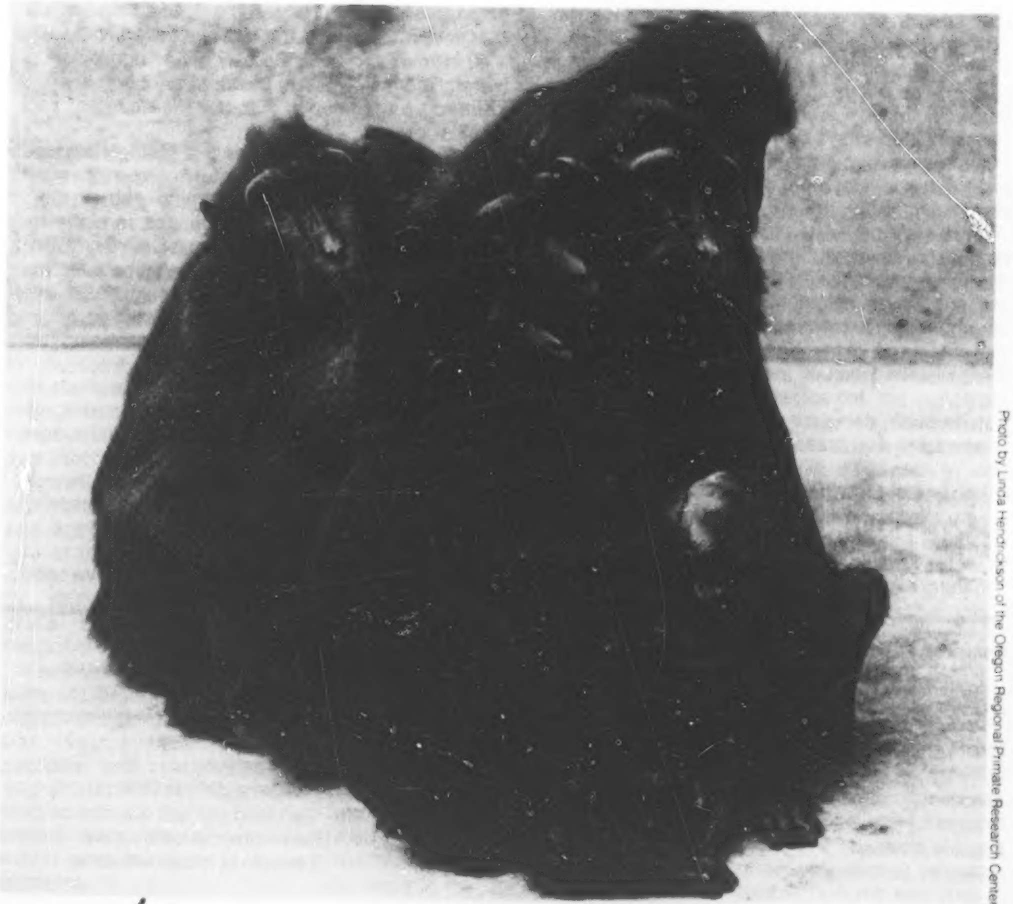

Photo by Linda Hendrickson of the Oregon Regional Primate Research Center.

Celebes macaques (*Macaca nigra*): *providing models for a type of diabetes mellitus that occurs in aging humans.*

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DIABETES *continued*

diabetes mellitus is exceptionally high in a colony of Celebes apes at the Oregon Regional Primate Research Center in Beaverton. More than half of the colony show prediabetic hormonal and metabolic abnormalities, while 10 to 15 percent show overt diabetes.

"Because most of the lesions found in the model also occur in humans and since the *M. nigra*—erroneously called an ape—is taxonomically close to man, the model constitutes a valuable research tool," says Dr. Charles F. Howard, Jr., of the Oregon center, who discovered the model.

Spontaneous diabetes mellitus in the animal is characterized by hyperglycemia, decrease of insulin, increase of glucagon, impaired glucose tolerance, and increased levels of serum lipids. Diabetic monkeys show weight loss, lethargy, and glycosuria. Atherosclerosis and cataracts are secondary complications. "The gradual inability to secrete insulin and to control glucose and lipid metabolism places the monkey in a metabolically stressed state," says Dr. Howard, "which over a period of years fosters the development of these complications."

Diabetes mellitus spontaneously develops in *Macaca nigra* due to a lesion in the islets of Langerhans. Slight hormonal changes and islet alterations can be measured early. Eventually there is a loss of secretory capacity due to secretory cell deterioration concurrent with a progressive and massive infiltration of the islets with amyloid. "The lack of functional beta cells is comparable to that which occurs in juvenile-type human diabetes and, in some instances, in the adult-onset type," Dr. Howard says. He also points out that the pathologic and hormonal changes in these monkeys permit detection of diabetes long before clinical diagnosis is possible.

The deposition of amyloid in the islets of Langerhans increases slightly with age in the normal *M. nigra*, but the increase was found to be much greater in monkeys with diabetes. (See *Research Resources Reporter*, April 1981, pp. 10-12). Since amyloid deposits also increase with age in humans, especially in older diabetics, Dr. Howard believes that "this phenomenon may contribute more to maturity-onset diabetes in aging individuals than has previously been realized."

The Chinese hamster (*Cricetulus griseus*) model, discovered in 1959, exhibits characteristics similar to those of humans with diabetes mellitus. Abrupt-onset diabetes mellitus in the model, as well as in man, is characterized by hyperglycemia, glycosuria, decreased pancreatic insulin, and initial hyperinsulinemia followed by a decline in serum insulin. Other similarities are failure of an insulin increase in response to glucose loading, abnormal liver metabolism, inheritance of the disorder, and decreased life expectancy. The model is also characterized by a lack of obesity, increased liver glycogen, and renal and hepatic gluconeogenesis.

The Chinese hamster model appears to be especially valuable for investigating early lesions and manifestations of diabetes mellitus, according to Dr. George C. Gerritsen of the Upjohn Company, Kalamazoo, Michigan. "It has become increasingly evident that glycosuria and hyperglycemia are late rather than initial manifestations of diabetes mellitus," says Dr. Gerritsen. "Because human diabetes is inherited, the unknown basic defect might be expected to occur at conception, if the proper genotype is present."

He points out that a study of prediabetes in man is difficult. Consequently, an animal model with the characteristics of human diabetes, such as the Chinese hamster, is needed for gathering information about this stage of the disorder.

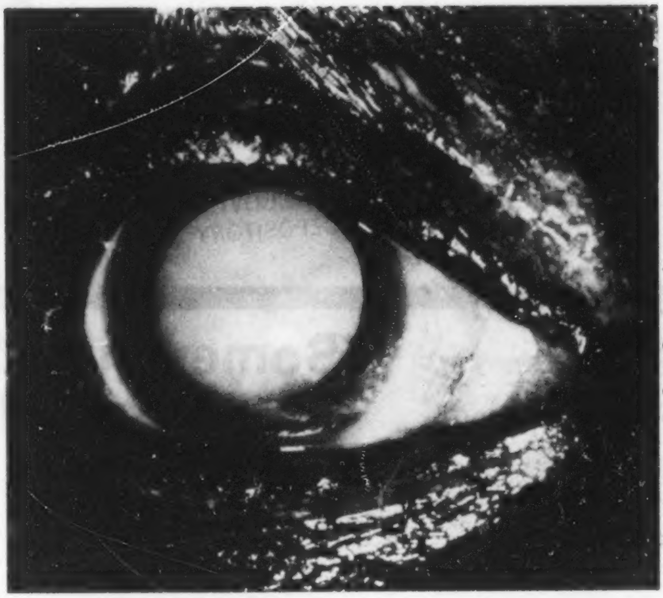

Photo courtesy of the Oregon Regional Primate Research Center

Surgical removal of a cataract from the eye of a severely diabetic monkey (left to right): *correcting a secondary complication of diabetes mellitus.*

Pathologic changes have been observed in the nervous, renal, and reproductive systems; the eye; and the pancreas of the diabetic Chinese hamster. Changes in the pancreas are degeneration, glycogen infiltration, and necrosis of the beta cells. The resultant decrease in beta cells may be due to genetic defects related to beta cell survival or replication, Dr. Gerritsen speculates.

The decrease in normally functioning beta cells results in the inability of the pancreas to synthesize or secrete insulin in response to a glucose stimulus. Increased gluconeogenesis in the liver also causes an ab-

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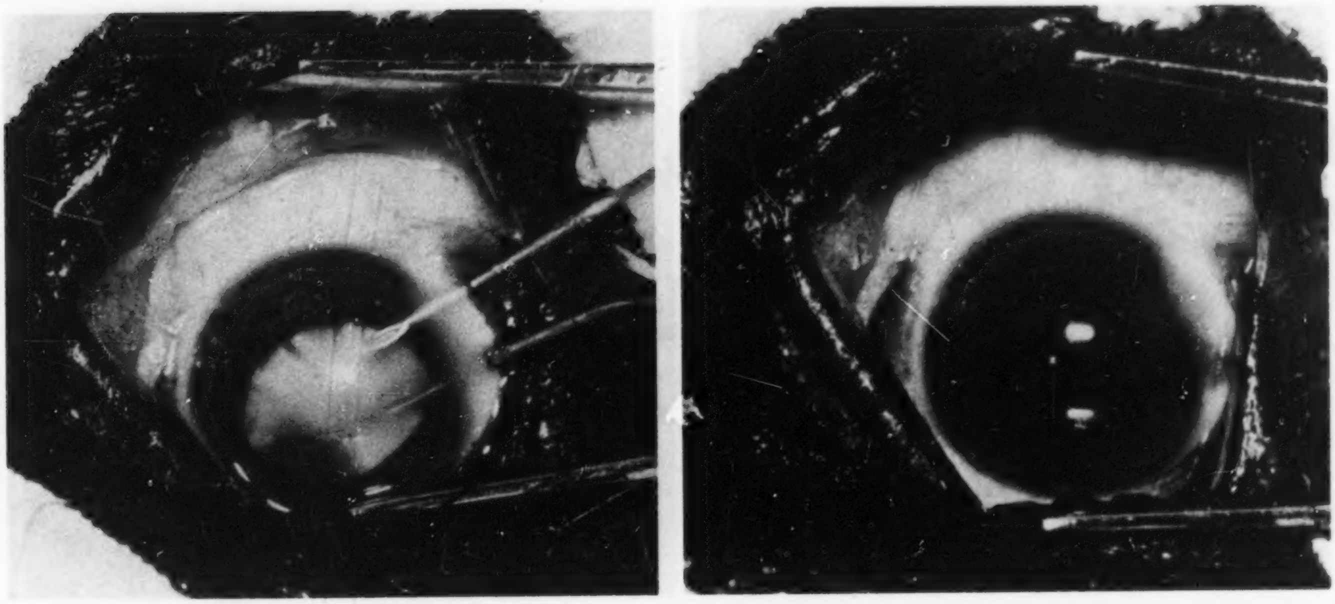


normally high output of glucose. These abnormalities produce the diabetic syndrome in the model.

Occasionally, spontaneous remission of diabetes has been observed in Upjohn's Chinese hamster colony. These remissions appear to be permanent and may be due to an effect of environmental factors on gene expression that normalizes metabolism.

In 1969 spontaneous diabetes mellitus was discovered in the South African hamster (*Mystromys albicaudatus*) by researchers at the University of Missouri Medical Center (UMMC) in Columbia. A diabetic colony was subsequently developed by Dr. Robert A. Stuhlman at UMMC through selective breeding. Currently there are no generally accessible sources of this diabetic animal.

The characteristics of diabetes mellitus in this model are similar in many respects to those in man, including beta cell lesions, hyperglycemia, glucose intolerance, polydipsia, polyuria, glycosuria, ketonuria, and genetic predisposition. Mild and moderate chronic syndromes corresponding to maturity-onset diabetes in the human occur in this model, as well as the acute or juvenile form. Secondary complications such as retinopathy, cataracts, glomerulosclerosis, and microangiopathy also are found. It is believed that spontaneous diabetes mellitus in the South African hamster has a polygenic mode of inheritance that is not sex linked.

"Hyperglycemia in *M. albicaudatus* varies in incidence, age at onset, degree of severity, rate of progression, and repeatability within individuals," states Dr. Stuhlman, now at Wright State University School of Medicine in Dayton, Ohio.

Lesions in the beta cells of the islets of Langerhans include beta degranulation, glycogen infiltration, vacuolization, nuclear pyknosis, and margination and degranulation of organelles. Studies indicate that the beta cell lesions vary directly with the extent and duration of hyperglycemia.

A valuable model of diabetes has been developed at the Pennsylvania State University's Laboratory Animal Diagnostic Resource in Hershey by Dr. C. Max Lang and his colleagues, following Dr. Lang's discovery in 1969 of a guinea pig that exhibited diabetes spontaneously. (See *Research Resources Reporter*, July 1979.) Onset of diabetes in the animal followed a viral infection. A group of guinea pigs has been found to be susceptible to a herpesvirus that is believed to trigger diabetes in the animals. Because of the prevalence of infection in the colony, the laboratory cannot provide guinea pigs to extramural investigators. Body fluids and tissues, however, are available.

Diabetes in the guinea pig mimics acute-onset diabetes mellitus in humans in many ways. The onset of juvenile-type diabetes occurs predominantly during puberty in both species. Also, early pathologic changes in the model are almost identical to those in the human pancreas. Insulin granules begin to disappear from beta cells in the islets of Langerhans, and the cells become depleted of the hormone.

Although there are many similarities, the guinea pig model differs from human diabetes mellitus in several important respects. First, children die from acute-onset diabetes if not treated with insulin. Guinea pigs do not. The animals recover after a crisis because they regenerate beta cells in the islets of Langerhans and gradually make enough insulin for a complete recovery. Beta cells are not regenerated in diabetic humans. Second, diabetes in the Hershey guinea pig model is attributed to the action of a virus so contagious that most of the more than 700 guinea pigs studied in the colony during the past 10 years contracted the infection and subsequently became diabetic. The diabetic animals have a defective cell-mediated immunity, according to Dr. Surej Singh, virologist at the resource. Evidence of a possible viral trigger of diabetes in humans was pro-

DIABETES *continued*

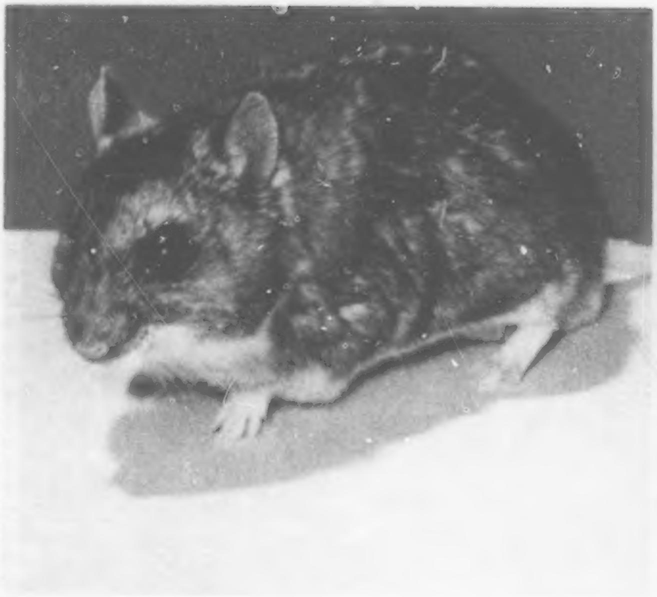


Photo courtesy Dr. Robert A. Stuhlman

South African hamster (*Mystromys albicaudatus*).

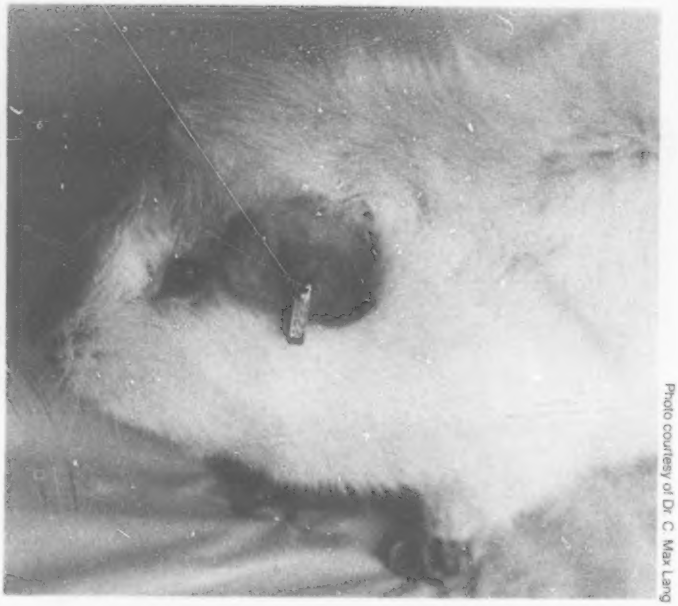


Photo courtesy of Dr. C. Max Lang

Diabetic guinea pig from the Laboratory Animal Diagnostic Resource in Hershey, Pennsylvania.

vided by a team of researchers from the National Institutes of Health and the National Naval Medical Center in Bethesda, Maryland, when they isolated from a diabetic boy a coxsackie B_4 virus variant, which caused symptoms of diabetes after it was injected into mice.

It is not known whether guinea pigs inherit susceptibility to diabetes as do humans. The Hershey researchers are collaborating with investigators at the University of Nebraska at Omaha and the University of Alabama at Birmingham to resolve this question.

The spontaneously diabetic biobreeding (BB) rat was discovered in 1974 by Drs. Reginald Chappel and Clifford Chappel in a commercial outbred colony of Wistar albino rats at the Bio Breeding Laboratories, Ltd., in Ottawa, Canada. A second major breeding colony of BB/Worcester (BB/W) rats has been established by Dr. Arthur Like at the University of Massachusetts Medical School in Worcester.

The animals develop spontaneous, insulin-dependent diabetes mellitus with symptoms and needs characteristic of the disease in man. Shared symptoms include increased water intake and elevated plasma glucose levels, glycosuria, hyperglycemia, lymphocytic thyroiditis, and insulitis with rapid destruction of islet beta cells and ketosis. "The BB rat is the only well-characterized model of insulinopenic diabetes in which the animals require daily administration of insulin to survive," says Dr. Like. Incidence of the spontaneous diabetes mellitus syndrome among various sublines ranges between 20 and 50 percent by 120 days of age.

Studies conducted by Drs. Like, Aldo Rossini, and Michael C. Appel strengthen the theory that the cause of diabetes in the BB/W rat is due to cell-mediated destruction of the pancreatic beta cells by an autoimmune mechanism associated with the thymus. Neonatal thymectomy reduced the incidence of diabetes from 19 to 4 percent. Treatment with antilymphocyte serum cured 36 percent of acutely ill diabetic rats and completely prevented diabetes in susceptible nondiabetic animals. Among the nontreated diabetic rats, 64 percent remained diabetic. Thirty-three percent of untreated normal animals became diabetic.

Studies showed that the incidence of diabetes in BB/W rats reared from birth in a germfree environment was not influenced by the absence of microorganisms. No microorganisms could be detected in the pancreas by electron microscopy at any stage of the disease.

Pathologic changes in the BB/W model consist of islet cell infiltrate of lymphocytes and macrophages, early beta cell necrosis, and complete destruction of the beta cells in the final stage of the disease. Lymphocytic infiltration of the thyroid is also frequently encountered among diabetic animals, suggesting the presence of a polyendocrine autoimmune disorder.

Researchers planning studies with the BB model are advised to have pathogen-free barrier facilities or laminar flow caging because the animals are very susceptible to *Mycoplasma* species and other respiratory tract pathogens.

The Egyptian sand rat (*Psammomys obesus*) is unique in that stress or a high-calorie diet induces a syndrome resembling diabetes mellitus. Even diets normally provided for laboratory animals cause the disorder. In its native habitat *P. obesus* has a low-calorie, high-mineral diet.

The model was discovered in 1964 by Drs. Knut Schmidt-Nielsen, Donald B. Hackel, and Harold E. Lebovitz and their colleagues at Duke University School of Medicine in Durham, North Carolina. There are no known sources of general supply of the model. Diabetes in the Egyptian sand rat may be chronic and relatively mild, or it may be acute and fatal. The chronic form is more like the maturity-onset type of diabetes in man, with obesity and hyperinsulinemia with or without hyperglycemia. The acute, fulminating disease is characterized by ketosis, acidosis, and early death. Animals bred in the laboratory more often develop the chronic,

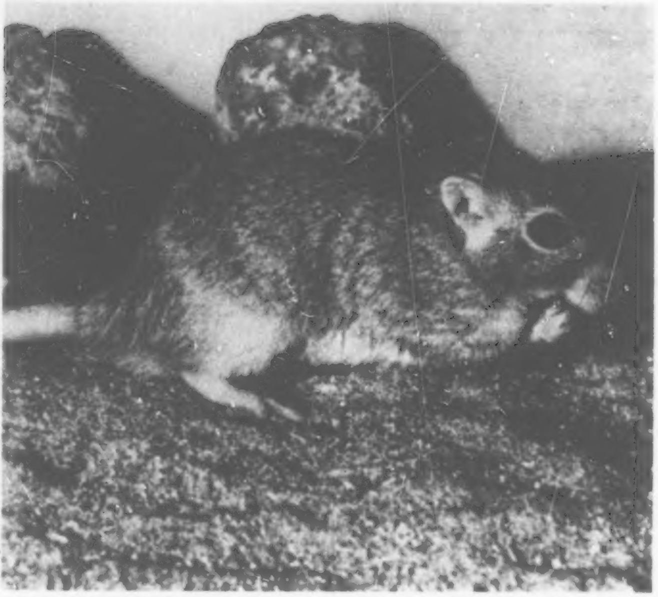


Photo courtesy of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

Egyptian sand rat (*Psammomys obesus*): *unique model of diabetes mellitus resulting from stress or a high-calorie diet.*

nonketotic type of the disease, whereas those captured in the wild and placed on a high-calorie diet usually develop the severe ketotic form.

The chronic, nonketotic type is characterized by a marked increase in plasma insulin and insulin resistance in the peripheral tissue. The acute, ketotic form is associated with insulin deficiency. The researchers point out that increased insulin secretion is the earliest and most dramatic change noted in the chronic form and that the elevated plasma insulin is related to the increased caloric intake. Increased glucose or other specific nutrients, however, have little if any effect on plasma insulin.

The diabetes syndrome in the model is believed to result from alterations in the environment such as high-calorie diet, stress, and infections. The nature of the genetic defect and the mechanism by which environmental factors induce glucose intolerance and ketosis are not known.

In vitro studies have revealed several other remarkable features of insulin secretion by the sand rat. First, the sand rat pancreas secretes considerably more insulin in vitro than that of most other species. Second, insulin secretion is not affected by stimulants such as glucose, tolbutamide, and dibutyl cyclic AMP or by inhibitors such as dopamine, which alters secretion in other species. Glucagon was the only agent studied that markedly stimulated in vitro insulin secretion by pancreatic cells of the sand rat. The researchers conclude from the results of these studies that insulin secretion by the sand rat is regulated by a series of unique controls.

Two well-established and well-characterized genetic mouse models of diabetes mellitus have been developed at the Jackson Laboratory in Bar Harbor, Maine, by Drs. Douglas Coleman and Edward Leiter and their colleagues.

The diabetes (*db*) model, caused by an autosomal recessive gene located on chromosome 4, is maintained in the C57BL/KsJ(BL/Ks) strain. The obese (*ob*) mutation, also an autosomal recessive mutation associated with chromosome 6, is now available in the C57BL/6J(BL/6) strain.

The *db* mouse is characterized by hyperphagia, transient insulinemia, marked hyperglycemia, severe diabetes, and premature death. A pronounced elevation of plasma insulin occurs as early as 10 days of age. Later the islets of Langerhans undergo hyperplasia and hypertrophy followed by atrophy. These pathologic changes are accompanied by a marked drop in the plasma insulin level.

Diabetes is mild in the *ob* mouse compared to the *db* model. It does, however, manifest marked obesity, hyperphagia, glucose intolerance, transient hyperglycemia, and pronounced elevated plasma insulin levels. Marked insulin resistance is associated with a loss of insulin receptors in several tissues. Hyperplasia and hypertrophy of islet beta cells occur instead of the beta cell destruction found in the *db* mouse.

The researchers believe that the loss of receptor sites in both models may protect the animals against the hyperglycemic effect of the greatly increased amount of circulating insulin. The *db* mouse is believed to have a defective satiety center. The *ob* mouse is believed to lack a satiety factor. The degree of obesity depends on the genetic background. Massive obesity results from a cycle of hyperinsulinemia leading to overeating, which then causes further secretion of insulin.

The severity of the diabetes syndrome produced by these mutant genes depends on an interaction with genes in the inbred strain background, the researchers say. Thus, both *db* and *ob* models with the BL/Ks inbred background are capable of interacting to produce a diabetes syndrome associated with beta cell necrosis, while in the diabetes-resistant BL/6 strain this does not occur.

The availability of congenic littermates permits tissue and organ transplantation without the danger of rejection, an important consideration when studying the effects of various tissues and organs on the development of diabetes.

Other diabetes models extensively discussed in the literature are the dog and the Yucatan miniature swine. Dog models are suitable for studying a wide variety of problems related to human diabetes. Swine models have dietary habits, gastrointestinal function, a tendency toward obesity, and a cardiovascular system that are similar to those in humans.

In the dog, spontaneous diabetes mellitus is estimated to occur with a prevalence between 1 to 200 and 1 to 800 in the general population of animals observed by veterinarians. Most cases occur in females over 6 years of age, and many are obese prior to onset of diabetes. Nearly all cases require insulin treatment.

A familial, early-onset type of diabetes mellitus in the golden retriever was recently reported. The offspring are not obese, onset of diabetes occurs between 2 and 5 months of age, and the animals require administration of insulin.

Blood serum, semen, and tissues that can be extracted without fatally damaging the animal are available from the Animal Model Center for Significant Human Diseases at Michigan State University in East Lansing. Slides on the histopathology of tissues from two diabetic golden retrievers are also available. According to Dr. George A. Padgett, principal investigator of the center, one goal of the resource is to eventually provide diabetic golden retrievers to researchers.

A genetically determined type of diabetes mellitus has been discovered in a line of nonobese keeshond dogs. Onset of the disease occurs between 2 and 6 months of age due to hypoplasia of the islets of Langerhans. The dogs do not grow unless treated with insulin.

Two lines of Yucatan miniature swine have been developed at Colorado State University in

DIABETES *continued*

Fort Collins. During gestation and lactation, a diabetic-like state and increased insulin resistance develop in some animals. Since 1972 the researchers have been selectively breeding the animals to develop a swine model with diabetic traits similar to those observed in man. One advantage of the pig is that researchers can assess fetal metabolism in utero while maternal metabolism is simultaneously monitored.

There are other advantages of the miniature swine model. It is large enough for the study of fetal metabolism but relatively small and hairless in contrast to domestic swine. It is docile, is easy to restrain in the laboratory, and does not become excited once it is familiar with the procedures used. Because it has a relatively long lifespan, longitudinal studies in individual animals can be conducted.

Disadvantages of the model are that most medical investigators are not familiar with pigs, their handling, and care. Long-term maintenance requires some specialized facilities, and maintenance costs for individual animals are high compared with those for care of rodents. They are, however, less than the costs of proper facilities and care for dogs or primates.

There is no known source of diabetic Yucatan miniature swine, although animals that may develop diabetes mellitus and pregnant animals that may develop gestational diabetes mellitus can be obtained from Colorado State University.

For information on the availability of animals or animal tissue for diabetes mellitus research, contact:

(Celebes apes)
Dr. Charles F. Howard, Jr.
Oregon Regional Primate
Research Center
505 Northwest 185th Avenue
Beaverton, Oregon 97006
Telephone (503) 645-1141

(Chinese hamsters)
Dr. George Gerritsen
Diabetes Research
The Upjohn Company
Kalamazoo, Michigan 49001
Telephone (616) 385-7075

(guinea pigs)
Dr. C. Max Lang
Milton S. Hershey Medical Center
Pennsylvania State University
500 University Drive
Hershey, Pennsylvania 17033
Telephone (717) 534-8462

(BB rats)
Dr. Pierre Thibert, Chief
Animal Resources Division
Sir Frederick W. Banting
Research Centre
Ross Avenue
Tunney's Pasture
Ottawa, Ontario
Canada K1A 0L2
Telephone (613) 995-7995

(BB/W rats)
Dr. Arthur A. Like
Professor of Pathology
University of Massachusetts
Medical School
55 Lake Avenue North
Worcester, Massachusetts 01605
Telephone (617) 856-3249

(*db* or *ob* mice)
Dr. Douglas Coleman
or
Dr. Edward Leiter
The Jackson Laboratory
Bar Harbor, Maine 04609
Telephone (207) 288-3371

(keeshond dog)
Dr. John W. Kramer
Department of Veterinary
Clinical Medicine and Surgery
Washington State University
Pullman, Washington 99164
Telephone (509) 335-8554

(golden retriever—tissues and slides)
Dr. George A. Padgett
Department of Pathology
Michigan State University
A19 Veterinary Clinical Center
East Lansing, Michigan 48824
Telephone (517) 355-0109

(Yucatan miniature swine)
Dr. Robert W. Phillips
Colorado State University
Fort Collins, Colorado 80523
Telephone (303) 491-5205
or
Ms. Linda Panepinto
Telephone (303) 491-8563

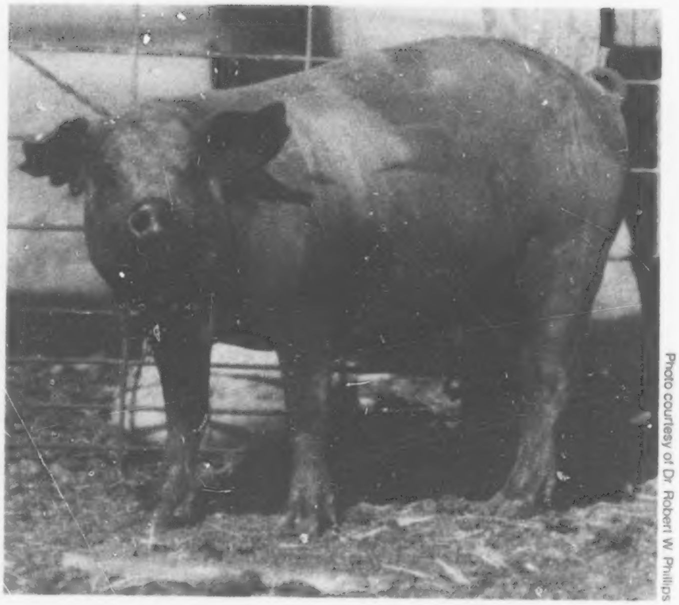


Photo courtesy of Dr. Robert W. Phillips

Yucatan miniature swine, 8-month-old, 60-pound female: *during gestation and lactation some animals develop a diabetic-like state with increased insulin resistance.*

Additional reading:

1. Howard, C. F., Jr., Spontaneous diabetes in *Macaca nigra*. *Diabetes* 21:1077-1090, 1972.
2. Gerritsen, G. C., and Dulin, W. E., Characterization of diabetes in the Chinese hamster. *Diabetologia* 3:74-84, 1967.
3. Stuhlman, R. A., Srivastava, P. K., Schmidt, G., Vorbeck, M. L., and Townsend, J. F., Characterization of diabetes mellitus in South African hamsters (*Mystromys albicaudatus*). *Diabetologia* 10:685-690, 1974.
4. Lang, C. M., and Munger, B. L., Diabetes mellitus in the guinea pig. *Diabetes* 25:434, 1976.
5. Like, A. A., Butler, L., Williams, R. M., Appel, M. C., and Rossini, A. A., Spontaneous autoimmune diabetes mellitus in the BB rat. *Diabetes* (in press).
6. Lebovitz, H. E., White, S., Mikat, E., and Hackel, D. B., Control of insulin secretion in the Egyptian sand rat (*Psammomys obesus*). *Diabetologia* 10:679-684, 1974.
7. Coleman, D. L., Inherited obesity-diabetes syndromes in the mouse. In *Mammalian Genetics and Cancer: The Jackson Laboratory Fiftieth Anniversary Symposium*. New York, NY, Alan R. Liss, Inc., 1981, pp. 145-158.

Research into the development of animal models of diabetes mellitus is supported in part by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases; the National Heart, Lung, and Blood Institute; the National Institute on Aging; the Animal Resources Program of the NIH Division of Research Resources; the Veterans Administration; the American Diabetes Association; Duke University; the University of Missouri Schools of Medicine and Graduate Education; the Medical Research Foundation of Oregon; the Colorado Affiliate of the American Diabetes Association; the Juvenile Diabetes Foundation; the Kroc Foundation; the Oregon Affiliate of the American Heart Association; the John A. Hartford Foundation; the Ruth/Vernon Taylor Foundation; the Becton and Dickinson Company; the Eli Lilly Company; the Pfizer Company, and the Upjohn Company. The Oregon Regional Primate Research Center, the Pennsylvania State University Laboratory Animal Diagnostic Resource, and the Animal Model Center for Significant Human Diseases are supported by the Animal Resources Program of the NIH Division of Research Resources.

Research Focus

New Method for Delivering Pancreatic Enzyme Supplements

A new system for administering supplemental enzymes has produced excellent results in a study of 23 patients with pancreatic enzyme insufficiency. The patients studied at the New England Deaconess Hospital in Boston, Massachusetts, were referred for the new treatment following surgery or after other forms of enzyme therapy failed.

The new oral delivery system, developed by Dr. George Blackburn of the Harvard Medical School, consists of porcine pancreatic extract molded into microspheres 2 to 3 mm in diameter that are coated with a pH-sensitive polymer and packaged into gelatin capsules.

Drs. Blackburn and David Valerio of the Harvard Medical School conducted the research, organizing and analyzing data with the PROPHET system, a national computer network available to more than 700 scientists n government, academia, and ndustry. (See *Research Resources Reporter*, June 1982, pp. 9-11.) The analysis indicates hat the microsphere enzyme treatment increases fat utilization by almost 50 percent more than does a placebo and reduces daily stool frequency to two per day from an average of five per day before treatment. The patients also gained an average of 4 kilograms in weight over a period of 1 year. One of the most gratifying findings, the researchers report, is that patient morale and quality of life improved. All those previously confined to their residences returned to work and to active social lives. No signs of toxicity or adverse clinical effects were noted during the trial.

The microsphere enzyme preparation produced particularly dramatic results in cystic fibrosis patients. "It not only helped them grow by aiding digestion of essential nutrients, but it also improved their lives socially by removing the stigma of frequent offensive bowel movements, Dr Blackburn says.

The principal causes of pancreatic enzyme insufficiency are alcoholic pancreatitis or surgery on the pancreas in the adult and cystic fibrosis in children. These patients lack the necessary enzymes for complete digestion of fats, carbohydrates, and

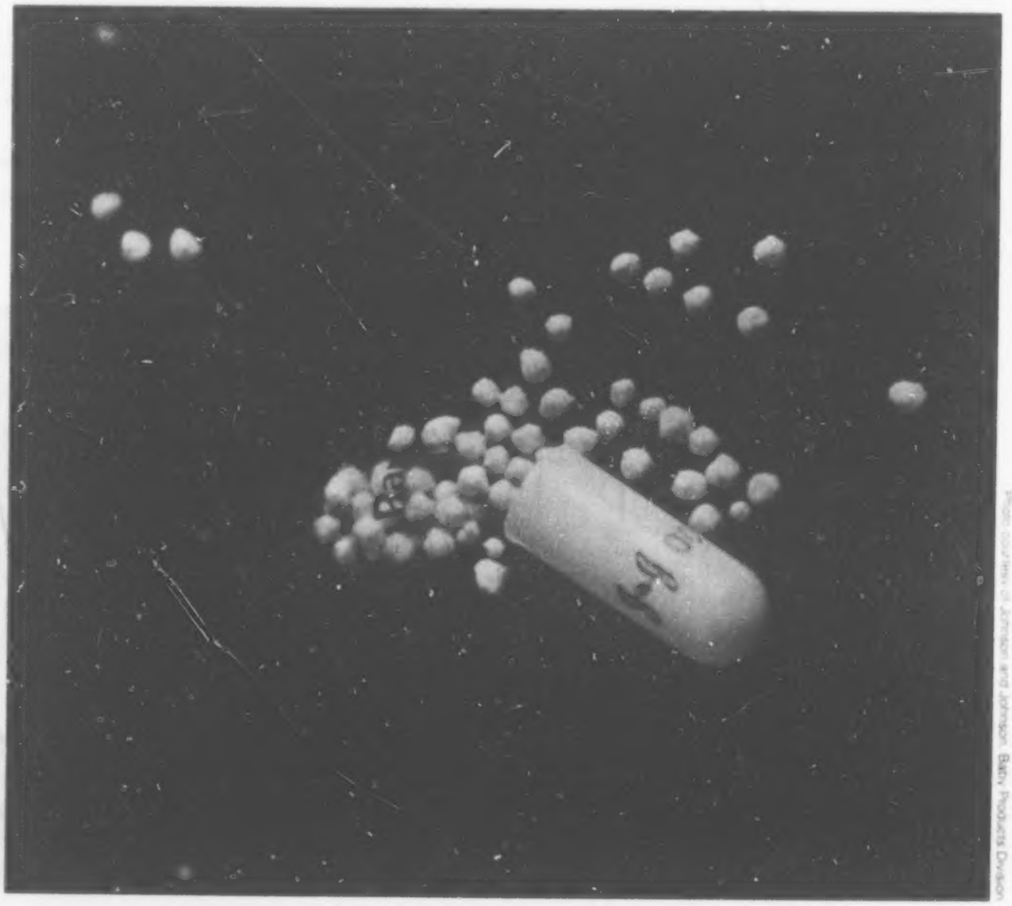


Photo courtesy of Johnson and Johnson, Baby Products Division

Gelatin capsule and microspheres: ***a new system for administering enzymes to children with cystic fibrosis and other patients with pancreatic enzyme nsufficiency.***

proteins.

The main problem in pancreatic enzyme insufficiency is the lack of the fat-splitting enzyme lipase. There is also a deficiency of amylase and protease, but Dr. Blackburn points out that the potential for digesting carbohydrates and protein exists in other parts of the digestive system, whereas pancreatic juice is the sole source of lipase. If lipase is absent, fats are not properly digested. Undigested fats are converted to soaps by bacteria in the colon. Because the average person takes in 50 to 100 grams of fat daily, frequent, bulky, offensive stools and abdominal cramps plague many patients, confining them to the home or at least restricting their social lives. Malnutrition may also result.

Treatment of pancreatic enzyme insufficiency requires lifelong oral administration of pancreatic enzyme supplements several times daily. A number of commercial preparations, mainly derived from swine pancreas, are currently available. Since pancreatic enzymes are quickly inactivated by acid in the stomach, various enteric-coated tablets have been developed. The main problems with this type of medication have been the large size of the tablets and their failure to release the enzymes before food passes through the jejunum.

In contrast, the microspheres pass easily through the stomach, releasing the enzymes quickly in the jejunum. Delivery is completed within 20 minutes after administration, says Dr. Blackburn.

In vitro tests revealed that the microspheres retained more than 97 percent of the enzyme activity after agitation for 1 hour in gastric fluid at pH 1.8. Approximately 90 percent of the enzymes were released within 8 minutes when the microspheres were exposed to artificial intestinal fluid at pH 7.5.

Because food fats are the primary cause of distress to patients with pancreatic enzyme insufficiency, Dr. Blackburn and his colleagues are trying to develop a fat that can be digested easily. They are focusing mainly on medium-chain triglycerides, which can be processed from coconut oil. These fats are more quickly broken down by digestive juices than the long-chain triglycerides, which comprise the bulk of the average daily fat intake.

Dr. Blackburn explains that polyglycerol esters can be synthesized to link the medium-chain triglycerides with long-chain essential fatty acids such as linoleic and linolenic acids. "We think that high-energy sources that are more easily digested will have broad application, providing patients who have cystic fibrosis and others who have pancreatic enzyme insufficiency a good source of fat without the problem of maldigestion," he says. "By combining the use of microsphere enzymes and the specially processed fats, we believe we'll be better able to manage this patient population."

Additional reading:

1. Valerio, D., Whyte, E. H. A., Schlamm, H. T., Ruggiero, J. A., and Blackburn, G. L., Clinical effectiveness of a pancreatic enzyme supplement. *Journal of Parenteral and Enteral Nutrition* 5:110-114, 1981.
2. Graham, D. Y., Enzyme replacement therapy of exocrine pancreatic insufficiency in man. *New England Journal of Medicine* 296:1314-1317, 1977.
3. Saunders, J. H. B., and Wormsley, K. G., Pancreatic extracts in the treatment of pancreatic exocrine insufficiency. *Gut* 16:157-162, 1975.

Research into microsphere enzyme treatment of pancreatic enzyme insufficiency is supported by the Biomedical Research Support Grant Program of the NIH Division of Research Resources, Johnson & Johnson Baby Products Company, and the Center for Nutritional Research Charitable Trust. The PROPHET system is supported by the Biotechnology Resources Program of the NIH Division of Research Resources.

Electron Microscopy Reveals Cancer Cell Changes

Electron microscopists have identified specific, abnormal surface features on bladder cells as indicators of cancer.

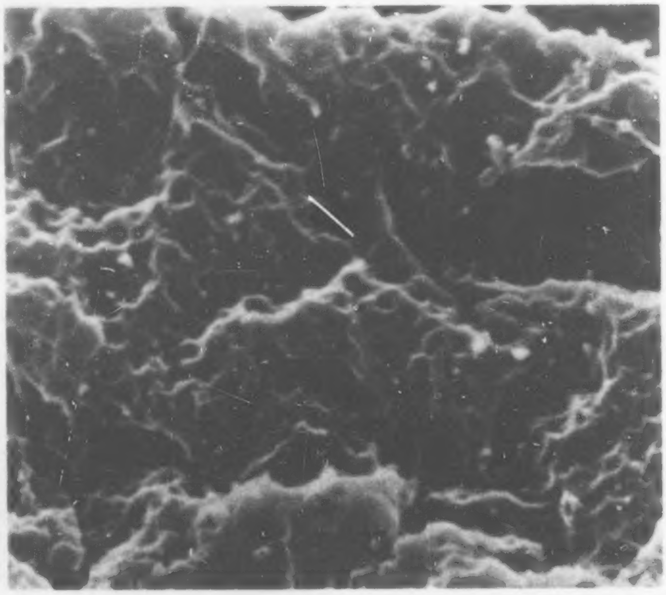
Images taken with a scanning electron microscope (SEM) reveal that the fingerlike projections called microvilli on the surface of human bladder cancer cells are pleomorphic, a state characterized by abnormal shape, size, or branching. In addition, the images show several types of cell-surface patterns apparently associated with bladder cancer. Variability of these patterns and changes in the degree of pleomorphism of the microvilli correlate with progressive stages of the cancer, according to the scientists.

The research by investigators at St. Vincent Hospital in Worcester, Massachusetts, and the nearby University of Massachusetts has increased the knowledge of how cells change as they progress from the earliest to the more advanced stages of the disease. The information may also be used to monitor patients at high risk of developing the cancer.

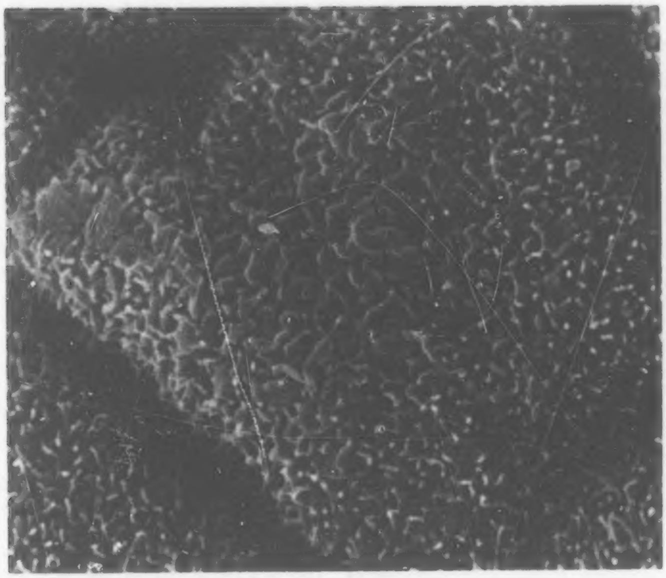
"For these patients, particularly those who have already presented with bladder cancer and are receiving treatment, SEM of bladder biopsies will be a reliable way of detecting recurrence of the disease or of following patient progress," says Dr. Jerome B. Jacobs, director of electron microscopy at St. Vincent Hospital and instructor of pathology at the University of Massachusetts Medical School. "The magnification needed with SEM is low, requiring relatively inexpensive instruments; these analyses can be done at less cost

The progressive stages of bladder cancer:

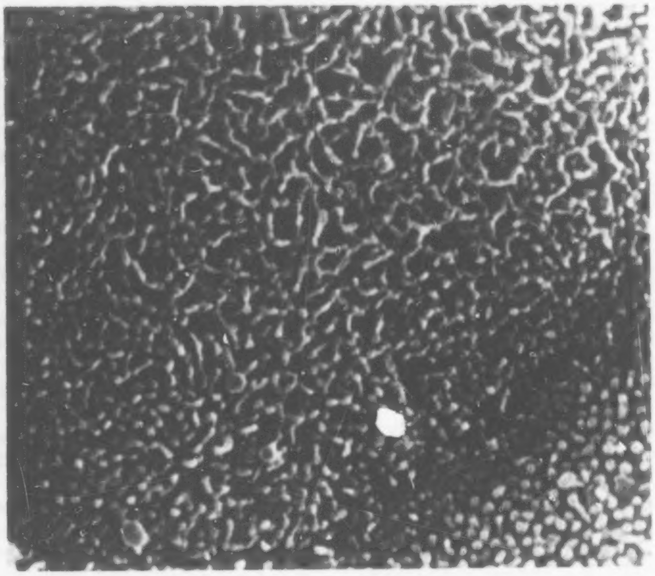
Normal (x14,000)



Information exchange

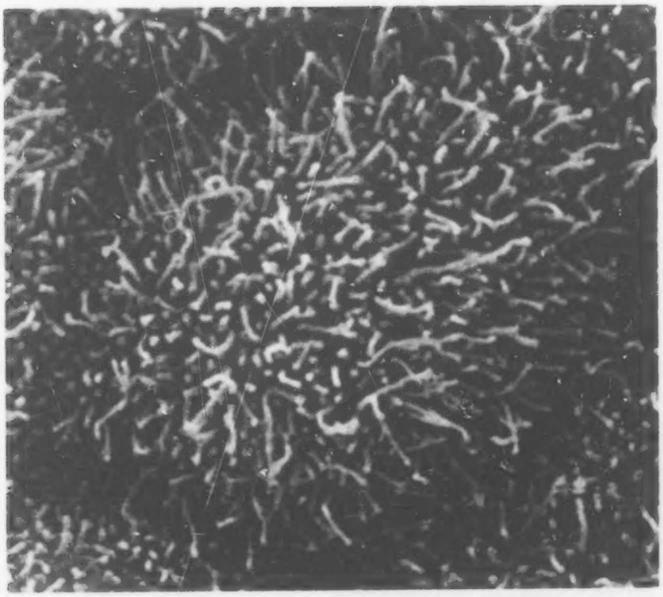


Type I (x6,600)

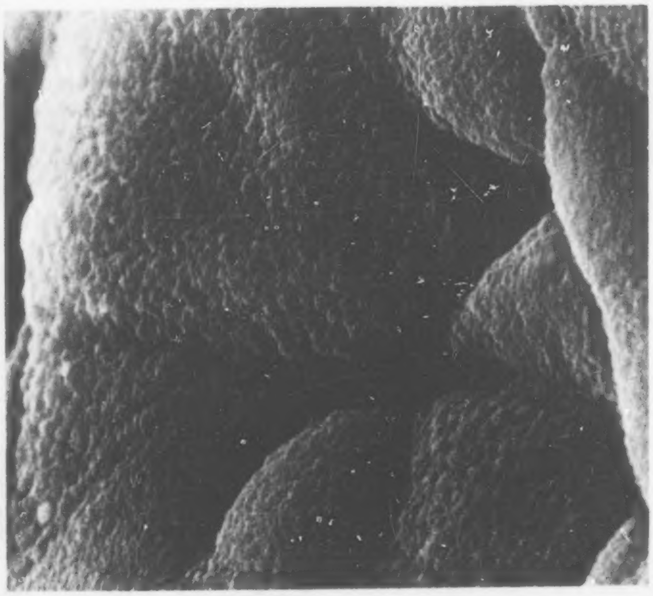


Type II (x9,300)

Type III (x8,600)



Type IV (x5,000)



than other procedures now in use. The SEM technique, however, is not appropriate for screening the general population," he says.

Dr. Jacobs and his colleagues at St. Vincent Hospital and the University of Massachusetts recently examined bladder biopsies from 17 patients with bladder cancer and 2 normal subjects, determining that pleomorphic microvilli, at the very least, indicate the presence of proliferating cells.

The presence of pleomorphic microvilli is one of several cell-surface features consistent with bladder cancer, says Dr. Jacobs. The degree of abnormality is related to the stage of cancer progression. The earliest stage is marked by cells of nearly normal size and shape with some pleomorphic microvilli. The number of these abnormal cells and the degree of pleomorphism and surface-pattern variance increase as the disease becomes more advanced or more aggressive. No pleomorphic microvilli are found in bladder cells taken from patients without cancer.

Researchers across the United States have identified pleomorphic microvilli in carcinomas of several organs other than the bladder, including the cervix, breast, colon, and liver. SEM examinations by Dr. Jacobs and others have documented that abnormal microvilli appear very early in the pathogenesis of experimentally induced cancer in animals. The researchers have also induced similarly pleomorphic microvilli in animals without cancer cells. "But the only way we could prove this difference and establish a marker for bladder cancer was to do a quantitative study of cancer patients, examining changes not only in microvilli but in the total cell surface," says Dr. Jacobs.

According to Dr. Jacobs, no quantitative studies of this type of pleomorphism in humans had been reported prior to the most recent study by his team, and the association between microvilli and the stage of tumor growth had not been explored in depth. "The beauty of SEM is that its technical procedures are fairly simple. A specimen can be quickly scanned for topographic features of the malignant cell," he says. "SEM analysis is a one- or two-person operation."

Dr. Jacobs and his colleagues found that microvilli are not present on normal luminal or surface bladder cells. Instead, these cells show microridges. Short microvilli do appear at junctions between cells of the intermediate and basal layers of the bladder. Their function is to keep the cells together, maintaining the integrity of the bladder.

Cancer cells imaged by the Jacobs research team show four basic patterns of variability. Cancer cells at early stages of progression are predominantly type I. Those in intermediate stages are mostly types II and III. Malignant cells at the most progressed stages of development are mostly type IV.

Type I cells are generally round and similar in size and shape. Uniform, short microvilli and ropy microridges mark the surface. Long microvilli are mostly unbranched. Cells have 1 to 20 pleomorphic microvilli, showing club-shaped and branched tips. Some cells are swollen and have scattered, short, pleomorphic microvilli. These cells appear to be dead and in the process of losing some surface features, particularly microvilli. In previous research, Dr. Jacobs and his colleagues determined that the loss of pleomorphic microvilli is common in apparently necrotic cells from the bladder.

Type II cells are round or oval, displaying between 20 and 50 pleomorphic microvilli. They have some uniform, short microvilli and occasional ropy microridges. These microridges do not have the asymmetric membrane plaques inserted in the luminal membrane of normal bladder cells. Some microridges appear beaded, a feature possibly caused by the fusion of microvilli, says Dr. Jacobs.

Type III cells, like types I and II, are generally round or oval but commonly have about 300 pleomorphic microvilli. They have few uniform microvilli and no microridges.

Type IV cells are strikingly abnormal, varying widely in size and shape. Dr. Jacobs divides type IV cells into four subclasses designated A through D. Subclass A is generally round or polygonal with no pleomorphic microvilli. The surface has uniform microvilli and thick, raised seams between cells. Subclass B cells have few or no pleomorphic microvilli and no uniform microvilli. The researchers hypothesize that the fusion of microvilli accounts for the corrugated or pebbly appearance of the entire cell surface. Seams between cells are thick. Subclass C cells are covered with more than 300 pleomorphic microvilli organized in clusters. Some microvilli are bent. Others are branched at the tips. Those with unbranched or straight tips are frequently bulbous or have teardrop shapes. Cells in subclass D are covered with pleomorphic microvilli that are generally shorter than those of the other subclasses. These microvilli are not clustered.

Dr. Jacobs believes that cell changes imaged with SEM are secondary features of internal control mechanisms that have gone awry in the cancer cell. He says further research will determine whether diseased, proliferating cells other than cancer cells show pleomorphic microvilli or other abnormal characteristics described in the recent study. It will also establish whether scanning electron microscopy of biopsy tissue can be used to evaluate the condition of patients with persisting or recurring bladder cancer.

Additional reading:

1. Jacobs, J. B., Cohen, S. M., Farrow, G. M., and Freidell, G. H., Scanning electron microscopic features of human urinary bladder cancer. *Cancer* 48:1399-1409, 1981.
2. Jacobs, J. B., The potential of scanning electron microscopic exfoliative cytology in the clinical management of human bladder cancer. In *Bladder Cancer, American Urological Association Monograph Series*, Baltimore, Md., Williams and Wilkins Co., 1982, vol. I, pp. 95-109.

The SEM study of human bladder cancer was supported by the National Cancer Institute and the Biomedical Research Support Grant Program of the NIH Division of Research Resources.

Research Resources Reporter
July 1982

Information Exchange

Research Highlights

A scanning agent to be used with gamma radiation imaging, including positron emission tomography (PET), has shown promising results for detecting malignant tumors, according to researchers at the Memorial Sloan-Kettering Cancer Center in New York.

"We believe that the scanning agent can be used as a biological probe to diagnose tumors noninvasively as well as to monitor tumor growth or regression after therapy," says Dr. Bernard Schmall, an organic chemist who synthesized the compound. "The agent has an excellent affinity for the variety of tumors that we've tested."

Dr. Schmall and his associates recently tested the synthetic amino acid called carbon-11-alpha-aminoisobutyric acid (AIB) on a patient with a malignant fibrous histocytoma in the right femur. The tumor rapidly absorbed and retained high concentrations of AIB and was clearly recognizable in the images. Healthy tissue surrounding tumor cells did not absorb the agent.

In previous animal studies, the researchers tested the compound on a dog with a naturally occurring adenocarcinoma and found that the agent had a high affinity for the tumor. Additional studies in dogs with lymphosarcoma or osteogenic sarcoma encouraged the researchers to use the agent for imaging human tumors.

Dr. Schmall says that AIB is incorporated rapidly into cancer cells and that because it is not metabolized after cellular uptake, it can be used to quantify differences in amino acid transport between normal and malignant cells without interference by subsequent metabolic products. The agent is safe for patients because it is cleared from the blood and tissues within a short time.

Carbon-11 is a cyclotron-produced, short-lived radionuclide that has a half-life of 20.4 minutes. Analysis with mass spectroscopy instruments available at the Rockefeller University's Mass Spectrometric Biotechnology Resource verified the structure of AIB, which was synthesized from unlabeled carbon compounds. The radionuclide emits positrons that collide with electrons in body tissue. Through this process, they produce gamma rays, which are detected and measured noninvasively by the PET scanner.

Dr. Schmall says that PET scans with the new agent may have advantages over conventional methods for diagnosing tumors. CAT and PET scans both provide three-dimensional images, but the PET scan also documents the biochemical and metabolic processes and the physiology of the tumor, allowing researchers to distinguish between normal and diseased tissue. "By comparing the concentration of the agent absorbed by the tumor before therapy with the concentration absorbed after therapy, we may determine if the tumor has continued to grow or has regressed," says Dr. Schmall.

He says the agent may be used to detect small brain tumors. Although AIB cannot cross the normal blood brain barrier, brain tumors apparently alter the barrier to allow access by the agent. Dr. Schmall plans to use the agent to detect primary and secondary brain tumors in patients.

The outlook for further clinical applications is excellent, according to Dr. Schmall. "We hope that the agent will be useful for in vivo tumor detection and that following chemotherapy, changes in AIB uptake by tumors may be correlated with effects of the treatment regimen," he says.

The researchers also are developing agents that may be useful in detecting breast cancer and other hormone-dependent tumors. Dr. Schmall has synthesized the estrogen derivatives ^{18}F-4-fluoroestradiol and ^{18}F-4-fluoroestrone, which are intended to bind with cell receptors of estrogen. He is attempting to develop these compounds with levels of radioactivity that will produce images distinguishing the tumor from surrounding tissues. "Since it has been shown that there is a direct relationship between the presence of estro-

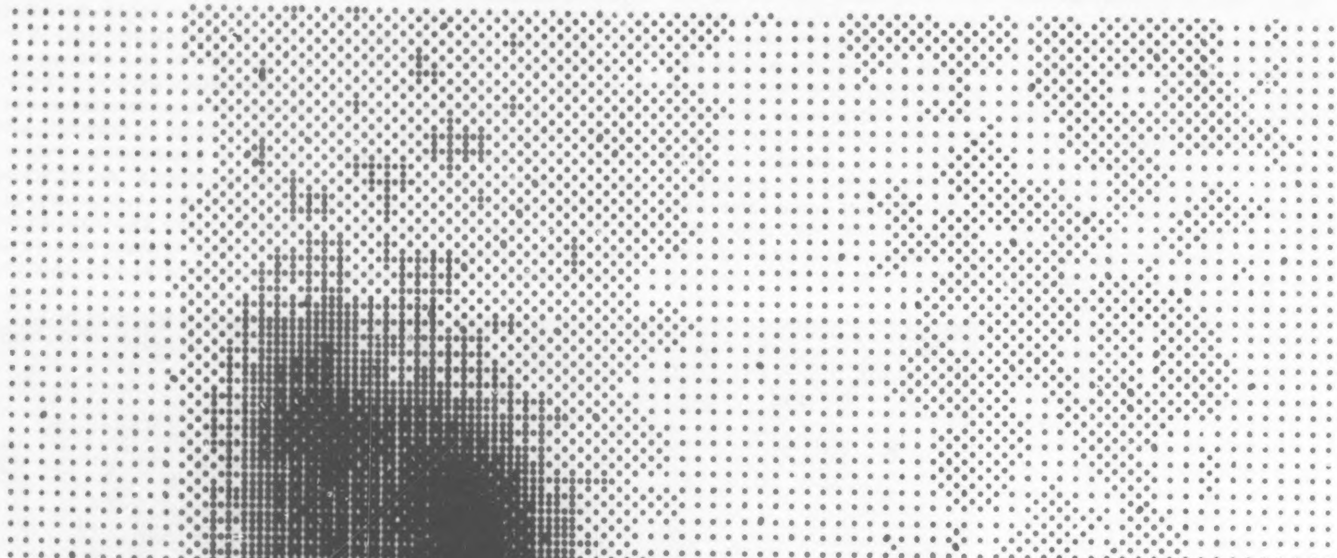
Dr. Bernard Schmall: *developing PET scanning agents for detecting tumors.*

gen receptors in malignancy and the remission of the tumor after endocrine therapy, the development of an estrogen derivative containing a positron-emitting radionuclide such as fluorine-18 may provide a noninvasive procedure for detecting estrogen-dependent primary and metastatic breast cancer," he says.

Additional reading:
1. Schmall, B., Bigler, R. E., Zanzonico, P. B., Conti, P. S., Dahl, J. R., and Lee, R., Tumor visualization and pharmacokinetic studies with C-11-alpha-aminoisobutyric acid (AIB) in dogs bearing spontaneous cancers. In *Proceedings of the World Federation of Nuclear Medicine and Biology's Third World Congress.* In press.
2. Schmall, B., and Bigler, R. E., Radiolabeled drugs: use of positron-emitting radionuclides. In *Radiopharmaceuticals: Structure-Activity Relationships* (Spender, R. P., ed.). New York, N.Y., Grune & Stratton, Inc., 1981, pp. 769-800.

Research by Dr. Schmall and his associates is supported by the Department of Energy, the National Cancer Institute, and the National Heart, Lung, and Blood Institute. The Mass Spectrometric Biotechnology Resource at Rockefeller University in New York is supported by the Biotechnology Resources Program of the NIH Division of Research Resources.

Gamma ray image of patient with malignant tumor in right femur (dark area): ***improving tumor detection with new scanning agent.***



Research Service

Histocompatibility Testing in Rats

A histocompatibility testing service for researchers using the rat as an experimental animal is now available through the International Committee on Laboratory Animal Science (ICLAS) Reference Center at the University of Pittsburgh. Dr. Thomas J. Gill III, professor and chairman of the pathology department, is director of the center, and Dr. Heinz W. Kunz, associate director.

The main purpose of the center is to provide histocompatibility typing services to investigators, to standardize typing reagents, and to check the genetic homogeneity of inbred and congenic strains of rats. Within its limits of capability, the center provides breeding nuclei of rats for researchers. Advice on the use of rats for various experimental purposes and on breeding and histocompatibility typing is also provided.

Many laboratory rats look similar. Without histocompatibility typing, researchers cannot be certain of strain identity or whether lots are genetically homogeneous. Dr. Gill points out that a great deal of effort and money have been wasted on studies using genetically impure animals. He hopes that services at the center will help prevent this waste of time and money and the generation of invalid research results.

The center has provided breeding nuclei of inbred and congenic strains of rats to approximately 100 investigators in the United States, Europe, and Asia. Histocompatibility typing services and reference antisera have also been provided to a large number of researchers.

Most requests for services of the center have come from researchers using the rat as an experimental animal in a variety of studies such as tissue transplantation, cancer research, reproductive biology, and immunobiology. A smaller number of requests have come from investigators directly involved in rat genetics.

There is a $50 handling charge for each breeding nucleus, plus $20 to $25 for a shipping carton that meets Federal specifications. The researcher also pays shipping charges. There is no charge for typing one or two specimens that can be worked into the regular histocompatibility typing schedule of the center. The fee is $50 for a larger number of specimens or those requiring a more complicated panel of testing. Dr. Gill points out that the center has no outside funding; the work is supported entirely by its research budget.

For further information about the center and its services, contact:

Dr. Thomas J. Gill III
Department of Pathology
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania 15261
Telephone (412) 624-2436
or
Dr. Heinz W. Kunz
Department of Pathology
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania 15261
Telephone (412) 624-2438

Questions and Answers

Q

Where can one obtain Rockland Swiss mice of breeding age?

Ms. Laura Harrington
Department of Biology
Vassar College
Poughkeepsie, New York 12601

A

Rockland Swiss mice of breeding age can be obtained for a fee from Rockland Farms, Inc. The approximate weight desired must be stated in the order, which may be placed by phone with purchase order number or by letter addressed to:

Rockland Farms, Inc.
P.O. Box 316
Gilbertsville, Pennsylvania 19525
Telephone (215) 369-1008

Q

What are the regulations regarding a research scientist from Japan bringing mice into the United States?

Dr. Hector Ramirez
Cleveland Clinic
9500 Euclid Avenue
Cleveland, Ohio 44106

A

The organizations that should be contacted regarding this matter are:

Centers for Disease Control
Biosafety Office
1600 Clifton Road, N.E.
Atlanta, Georgia 30333
Telephone (404) 329-3883

U.S. Department of Agriculture
Import-Export Staff
Veterinary Services Division
6505 Belcrest Road
Lanham, Maryland 20782
Telephone (301) 436-8170

U.S. Fish and Wildlife Service
Federal Permit Office
P.O. Box 3654
Arlington, Virginia 22203
Telephone (703) 235-1903

Handbook on Computer Intelligence Published

The first volume of *The Handbook of Artificial Intelligence*, a compendium of knowledge concerning computer intelligence techniques and their applications in diverse fields ranging from education to medicine, is now available. The second and third volumes are scheduled to be published in June 1982.

The compendium presents the broad scope of techniques and concepts in artificial intelligence (AI). It fills the gap between highly technical articles written for experts in AI and the overly simplistic accounts intended for the general public. The articles can be understood by persons with no AI background.

When completed, the handbook will contain more than 200 short articles covering the important ideas, techniques, and systems developed during 25 years of AI research. Some articles serve as overviews, discussing the various approaches within subfields and the problems inherent in these areas.

The articles are short. They begin with general descriptions and become progressively detailed, allowing readers to choose the depth to which they want to pursue a particular topic. Each article has been edited to remove confusing and nonessential jargon and each will be revised as the field of AI grows.

The handbook was conceived in 1975 by Dr. Edward A. Feigenbaum, director of the Stanford University heuristic programming project and principal investigator of SUMEX-AIM (Stanford University Medical Experimental Computer-Artificial Intelligence in Medicine), a nationwide network of researchers pursuing the application of AI techniques in biomedicine. SUMEX-AIM is supported by the Biotechnology Resources Program of the NIH Division of Research Resources. Dr. Feigenbaum edited the handbook with Mr. Avron Barr and Mr. Paul Cohen, doctoral candidates in the Stanford computer science department.

Hundreds of computer science researchers in the SUMEX-AIM network helped the editors write, edit, and check the accuracy of articles in the handbook. Dr. Feigenbaum says the cooperation, which occurred via computer-telephone links or "electronic mail," demonstrates how a network such as SUMEX-AIM can improve communication in the scientific community.

For more information on *The Handbook of Artificial Intelligence*, write to:

William Kaufmann, Inc.
One First Street
Los Altos, California 94022

RRIC Publications Win Awards

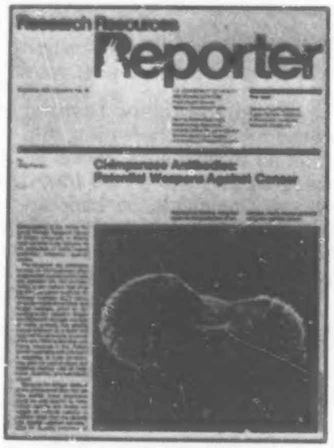
Two publications of the NIH Division of Research Resources (DRR) have received awards for excellence in biomedical publications.

First prize was awarded by the Mid-Atlantic Chapter of the American Medical Writers Association (AMWA) to the Research Resources Information Center (RRIC), a service of DRR, for the article "Chimpanzee Antibodies: Potential Weapons Against Cancer." The article was printed in the December 1981 *Research Resources Reporter*. The award, given for periodical articles intended for a professional audience, was presented at the annual meeting held May 26, 1982, in Washington, D.C.

Third prize was presented to RRIC in a national competition of the Health Sciences Communications Association (HeSCA) for the *Animal Resources Directory*, 1981 edition. The prize, which was awarded in the newsletter/brochure category for general circulation publications, was presented during a ceremony held May 25, 1982, at the annual meeting in San Antonio, Texas.

RRIC serves as a channel of communication between the five DRR programs covering animal resources, general clinical research centers, biotechnology resources, minority biomedical research support projects, and biomedical research support grants.

The *Reporter* article cited by AMWA describes the ongoing development of monoclonal antibodies for possible use in diagnosis and treatment of cancer patients. The studies being done by researchers at Duke University involve chimpanzees housed and cared for at the DRR-supported Yerkes Regional Primate Research Center in Atlanta, Georgia. The directory cited by HeSCA lists and describes shared research facilities in the DRR Animal Resources Program.



Meetings

International Epilepsy Symposium to Be Held in 1983

The 15th Epilepsy International Symposium, cohosted by the American Epilepsy Society and the Epilepsy Foundation of America, will be held September 26 to 30, 1983, in Washington, D.C.

The symposium will address such wide-ranging topics as epilepsy and employment and the influence of government on persons with epilepsy. Program themes include surgery, PET scanning, neurotransmitters and peptides, and the relationship of epilepsy with pregnancy. The binding of antiepileptic drugs will also be discussed.

Cochairmen for the gathering will be Dr. Roger J. Porter, chief of the epilepsy branch of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and Dr. Arthur A. Ward, Jr., an NINCDS grantee at the University of Washington in Seattle. Ms. Nyrma Hernandez, a member of the NINCDS council, will serve on the symposium's advisory committee.

For more information on the 1983 symposium, contact:

Driscoll and Associates
1925 North Lynn Street
Suite 1002
Arlington, Virginia 22209
Telephone (703) 522-5202

Symposium on Medical Imaging

The first IEEE Computer Society International Symposium on Medical Imaging and Image Interpretation, ISMIII '82, will be held in Berlin, Germany, October 26 to 28, 1982. This symposium will provide a transdisciplinary forum for biomedical and computer scientists, engineers, and medical professionals to discuss recent technological advances in biomedical imaging and signal acquisition and recent innovations in computer architecture and displays. The symposium will focus on biomedical image, signal, and pattern analysis and computer graphics.

For more information, contact:

Dr. Judith M. S. Prewitt
Division of Computer Research and Technology
Office of the Director
Building 12A, Room 2053
National Institutes of Health
Bethesda, Maryland 20205
Telephone (301) 496-1247

13th International Cancer Congress

The 13th International Cancer Congress to be held in Seattle, Washington, September 8 to 15, 1982, will provide an update on preclinical and clinical oncology and allied disciplines. Major emphasis will be given to significant advances in clinical investigations since 1978, progress in understanding the mechanisms of cell growth control and cell differentiation, identification of biological responses that define tumor-host relationships, progress in clarifying the mechanisms of chemical carcinogenesis and the genetic and environmental factors involved in the development of cancer, patient care and its organization, development of new therapeutic approaches, and progress in managing neoplastic diseases.

For additional information, contact:

Dr. Edwin A. Miran
Secretary-General
Congress Operations Office
13th International Cancer Congress
Suite 1800
Fourth and Blanchard Building
Seattle, Washington 98121
Telephone 1 (800) 426-9920

Symposium on Cellular Aging in the Vascular System

The W. Alton Jones Cell Science Center will hold a symposium on "Cellular Aging in the Vascular System: In Vitro and In Vivo Approaches," August 16 to 18, 1982, in Lake Placid, New York. Topics to be covered include in vivo and in vitro investigation of normal structure and function, characteristics of senescence, and pathology of the major cells that comprise the vascular system. The meeting will consist of a 2-day series of formal presentations by invited speakers and a half-day session of short, submitted and invited papers and poster sessions. Registration fee is $25.

For additional information, contact:

Dr. James R. Smith
W. Alton Jones Cell Science Center
Lake Placid, New York 12946
Telephone (518) 523-2427

July 18-23, 1982

Title: *International Conference on Animal Blood Groups and Biochemical Polymorphisms*
Location: *Ottawa, Canada*
Contact: *Dr. D. T. Colling*
Animal Disease Research Institute
801 Fallowfield Road
P.O. Box 11300
Station "H"
Negean, Ontario
Canada K2H 8P9

July 19-24, 1982

Title: *European Cell Biology Organization Congress*
Location: *Paris, France*
Contact: *Circle Francaise de Biologie Cellulaire*
67 Rue Maurice Gunsbourg
F-94200 Iyry-sur-Seine, France

August 8-13, 1982

Title: *IUMS International Congress on Microbiology*
Location: *Boston, Massachusetts*
Contact: *R. W. Sarber*
American Society for Microbiology
1913 I Street, N.W
Washington, D.C. 20006
Telephone (202) 833-9680

August 9-13, 1982

Title: *Lasers, Microwaves, Ultraviolet Rays, and Ultrasound: Biophysical and Biological Basis, Applications, and Hazards in Medicine and Industry*
Location: *Cambridge, Massachusetts*
Contact: *Director of Summer Sessions*
Room E19-356
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
Telephone (617) 253-2101

August 10-13, 1982

Title: *Symposium on Respiratory Distress Syndrome*
Location: *Helsinki, Finland*
Contact: *Dari Raivio*
Helsinki University Central Children's Hospital
Stenbackinkatu 11
SF-00290 Helsinki 29, Finland

August 26-28, 1982

Title: *Advances in Diagnostic Imaging*
Location: *San Francisco, California*
Contact: *Radiology Postgraduate Education*
University of California, San Francisco
Room M396
Third and Parnassus Avenue
San Francisco, California 94143
Telephone (415) 666-5731

August 30-September 3, 1982

Title: *International Symposium on Recent Developments in Perinatal and Childhood Infection*
Location: *Jerusalem, Israel*
Contact: *M. Isacsohn*
Organizing Committee
P.O. Box 983
Jerusalem 91009, Israel

September 15-17, 1982

Title: *Biological Actions and Medical Applications of Dimethyl Sulfoxide (DMSO)*
Location: *New York City, New York*
Contact: *Conference Department*
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021
Telephone (212) 838-0230

September 20-22, 1982

Title: *2nd International Meeting of the Bioelectrical Repair and Growth Society*
Location: *Oxford, England*
Contact: *Betty F. Sisken, Ph.D.*
Wenner Gren Research Laboratory
University of Kentucky
Lexington, Kentucky 40506
Telephone (606) 258-5796

September 23-25, 1982

Title: *MEDCOMP '82: First IEEE Computer Society International Conference on Medical Computer Science/Computational Medicine*
Location: *Philadelphia, Pennsylvania*
Contact: *Judith M. S. Prewitt, Ph.D.*
Division of Computer Research and Technology
Building 12A, Room 2053
National Institutes of Health
Bethesda, Maryland 20205
Telephone (301) 496-1247

October 3-8, 1982

Title: *33rd Annual Session of the American Association for Laboratory Animal Science*
Location: *Washington, D.C.*
Contact: *Joseph Garvey*
The American Association for Laboratory Animal Science
Suite 205
210 North Hammes Avenue
Joliet, Illinois 60435
Telephone (815) 729-1161

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Research Resources
Reporter

August 1982, Volume VI, No. 8

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AND HUMAN SERVICES
Public Health Service
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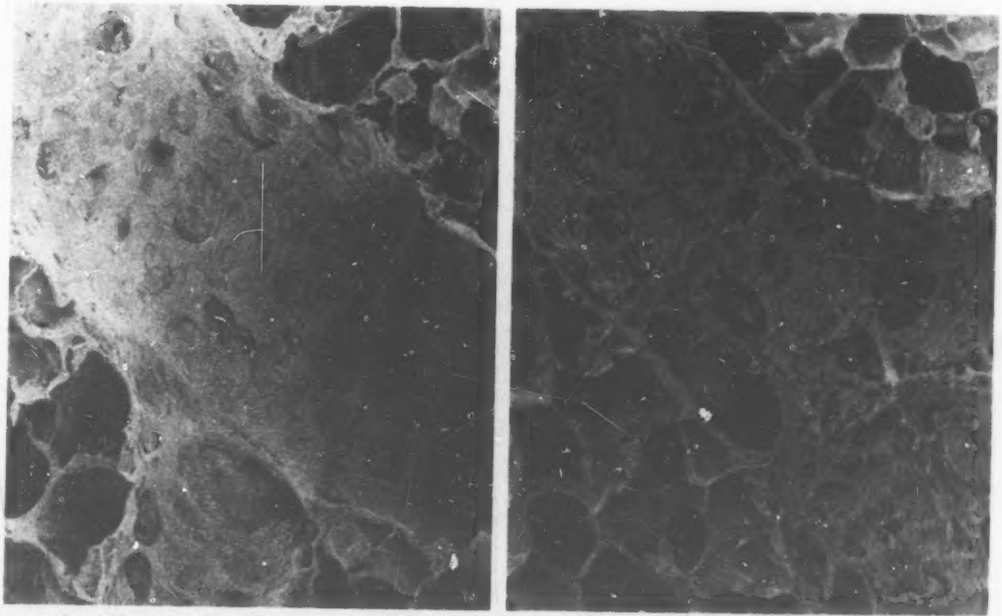
Serving Animal Resources,
Biotechnology Resources,
General Clinical Research Centers,
Minority Biomedical Research Support,
and Biomedical Research Support

This Issue:

Visual Field Disorders
Preventing Premature Labor
Cytomegalovirus Vaccine
Measuring Ciliary Activity

by
Gail Shiner Makulowich

Primate Center Investigates Health Effects of Photochemical Smog



Scanning electron micrographs showing a normal small airway of a bonnet monkey (left) and a small airway of a bonnet monkey exposed to 0.64 ppm of ozone for 8 hours daily for 90 consecutive days: ***exposed airway (right) shows cell proliferation and thickening of the airway lining.***

Heavy smog can cause impaired lung function, nausea, headache, increased susceptibility to infection, and other symptoms in healthy people. Impaired lung function is usually not serious in healthy people, as long as they are not engaged in heavy exercise. Even slight lung impairment, however, may be dangerous to at-risk populations, such as those with asthma, emphysema, and other pulmonary problems.

The most damaging pollutant of photochemical smog is ozone. Its concentration rose to 0.5 parts per million (ppm) in the atmosphere during a full-stage smog alert in Los Angeles last summer. Ozone concentration reaches 0.2 ppm on most summer days in the severely polluted areas of Los Angeles. A similar level of ozone concentration also occurs on some summer days in the cities of Washington, Boston, Denver, and Houston, where heavy traffic and industrial pollutants chemically interact with sunlight to form ozone and other photochemical products. The National Ambient Air Quality Standards required by the Clean Air Act define the maximum allowable concentration of ozone to be 0.12 ppm, a limit that some suggest should be raised to 0.2 ppm.

Several groups of researchers at the pollution exposure facility of the California Primate Research Center in Davis have been investigating the health effects of low levels of ozone (0.1 to 0.64 ppm) for many years. Their recent research indicated that exposure of rats and monkeys to 0.16 ppm of ozone caused slight but obvious damage to the small airways of the lung. Exposure of these animals to 0.64 ppm of ozone caused lung inflammation and fibrosis, which can reduce lung elasticity and breathing capacity, and caused abnormal lung growth in young monkeys. Exposure to 0.4 ppm of ozone caused immunological changes in the lung that can exacerbate allergies and asthma.

Dr. Donald L. Dungworth, di-

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SMOG, *continued*

rector of respiratory disease research at the primate center; Dr. Walter S. Tyler, veterinarian and professor of anatomy, University of California at Davis; and Dr. Lester W. Schwartz, veterinary pathologist and former member of the research unit team, have investigated the inflammatory effects of ozone. In separate studies of rats and monkeys, they found inflammation of the lung as a result of exposure to 0.16, 0.4, or 0.64 ppm of ozone for periods ranging from 7 to 360 days. Exposure to these ambient ozone levels causes most damage to the acinar areas of the lung, according to Dr. Dungworth. These regions, which are critical to the efficient exchange of oxygen and carbon dioxide, are located at junctions between the small airways called bronchioles and the small air sacs called alveoli.

The inflammatory effect of ozone is demonstrated by changes in the epithelial cells that line the lung and by the increased numbers of alveolar macrophages, the lung's main defensive cells, that respond to substances released by the interaction of lung cells and liquids with ozone. Exposure to low levels of ozone damages or destroys the ciliated cells lining the small airways. These cells have hairlike projections called cilia that help clear harmful particles from the lung. When destroyed by ozone, they are replaced by nonciliated cells that are more resistant to damage. In the alveoli, flattened type 1 epithelial cells that are very susceptible to damage by ozone are replaced by more compact and less susceptible type 2 cells. Accumulation of macrophages, proliferation of cells lining the bronchioles, and inflammation in the bronchiolar walls can cause narrowing of the small airways, which impairs breathing capacity, says Dr. Dungworth.

"The abnormalities that result from exposure to approximately 0.2 to 0.4 ppm of ozone are not readily detectable by pulmonary function tests," he adds. "At these low levels, chronic lung damage in monkeys is essentially limited to the small airways. This damage is similar to that seen in the small airways of young smokers. Because slight changes in the small airways are not easily detected by pulmonary function tests, there can be gradual damage for a long time before impaired lung function is noticeable."

Studies in both monkeys and rats have shown that cells lining the lung can adapt to low concentrations of ozone, asserts Dr. Dungworth. This is indicated particularly by reduced inflammation with continued exposure. For example, fewer alveolar macrophages accumulated in the small airways of bonnet monkeys after 90 days of exposure to 0.64 ppm of ozone than in the small airways of monkeys exposed for only 7 days. Epithelial cell changes and macrophage accumulation also occurred in the lungs of rats exposed to 0.16 ppm of ozone for 7 days. After 90 days of exposure, however, the lungs of controls and exposed rats were virtually indistinguishable.

"Adaptation is a widely used term in biology," explains Dr. Dungworth. "Unless it is defined precisely, it can be a source of confusion. We use it at the cellular level to refer to changes in cells at risk of ozone injury. These changes make them less susceptible to damage than they were in their naive state. Little is known about the mechanisms of adaptation to ozone, but preliminary work at Davis and elsewhere indicates that it is brought about by shifts in cell populations as well as changes within cells. The extent to which adaptation is protective at the concentration of ozone common in severe photochemical smog (0.2 ppm) still has to be determined by long-term exposure of monkeys."

Two factors must be considered in evaluating damage caused by chronic exposure to ozone or other pollutants. One is the intensity of inflammation, or rate of damage at any point in time; the other is the cumulative damage up to that point. Evidence of adaptation has been drawn mostly from indicators of intensity of inflammation, such as proliferation of alveolar macrophages and changes in epithelial cells. "Prediction of long-term damage by ambient levels of ozone cannot be made accurately at the moment," explains Dr. Dungworth. "We need long-term studies to assess the relationship between the ongoing rate of damage and cumulative damage at various intervals. We need to correlate markers of cumulative damage with markers of intensity of inflammation such as the number of inflammatory cells, the quantities of mediating substances, the rate of epithelial cell proliferation, and the rate of collagen synthesis. Cumulative damage is indicated by structural changes in air spaces, altered appearance of the epithelium, and the total amount of collagen. It is changes in the epithelium and the collagen that produce clinical effects that are generally irreversible."

Collagen is a protein present in connective tissue fibers. It is used as a marker of chronic damage because collagen-producing connective tissue cells proliferate at the site of chronic inflammation. The relationship between changes in lung collagen content and the occurrence of pulmonary fibrosis is still being actively investigated, according to Dr. Jerold A. Last, associate professor of medicine at the University of California at Davis and associate research biochemist at the primate center. He and Dr. Karen M. Reiser, assistant research biochemist, University of California at Davis, and their colleagues found that exposure to high concentrations of ozone increased the rate of lung collagen synthesis and induced pulmonary fibrosis—accumulation of connective tissue in the lung—in previously healthy rats. The level of fibrotic response and corresponding reduced lung elasticity depended on the concentration of ozone to which the rats were exposed, which ranged from 0.64 ppm to 1.2 ppm.

"Collagen is the major structural component of the lung, comprising 15 percent of the lung's dry weight," explains Dr. Last. "It is the scaffolding on which the lung is built and it is responsible for some of the lung's mechanical properties. There are still controversy and speculation about the role of collagen in the lung. Nevertheless, collagen and elastin, another connective tissue protein, seem to account for the elastic properties

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of the lung and the ability of alveoli to expand and contract."

In one study, Dr. Last and his colleagues found that exposure to 0.64, 0.96, and 1.2 ppm of ozone altered the type of collagen being synthesized in rat lungs. They found that more type 1 than type 3 collagen was synthesized. Type 1 collagen is stiffer than type 3 collagen and potentially restricts expandability of the alveoli. The same study also showed an increase in the rate of lung collagen synthesis following exposure to ozone. Rat lungs exposed to either 0.64 or 0.96 ppm of ozone showed double the normal rate of collagen synthesis and rat lungs exposed to 1.2 ppm of ozone showed a threefold increase. In a previous rat study, Dr. Last demonstrated that detectable increases in the rate of lung collagen synthesis preceded discernible tissue fibrosis by as much as 7 days.

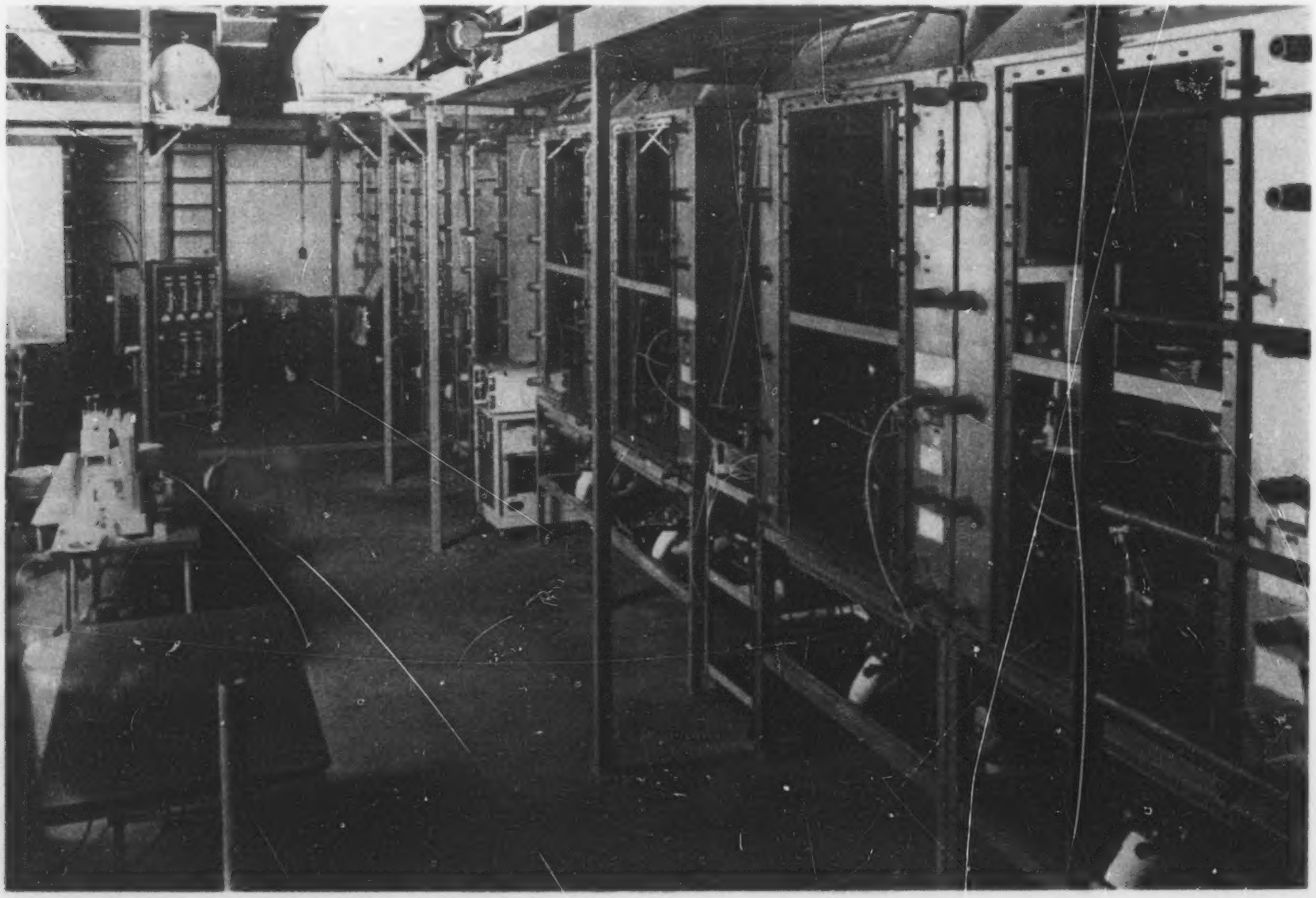
Dr. Last cautions that short-term exposure of rats and monkeys to high ozone concentrations cannot be extrapolated to long-term exposure of humans to ambient levels of ozone. "We need studies of chronic exposure to see if subtle injury to the lung follows continuous exposure to low levels of air pollution," he says. "Current air quality standards are necessarily based on data derived from short-term studies, because very few long-term studies have been done."

He explains that rats are of only limited value in assessing the effects of oxidant air pollutants on humans because of fundamental differences in lung anatomy and breathing patterns. The rat is an obligate nose breather. Moreover, rats, unlike monkeys and humans, do not have terminal bronchioles, the areas of transition between the small airways and the alveoli. In the rat, the small airways lead directly into alveolar ducts.

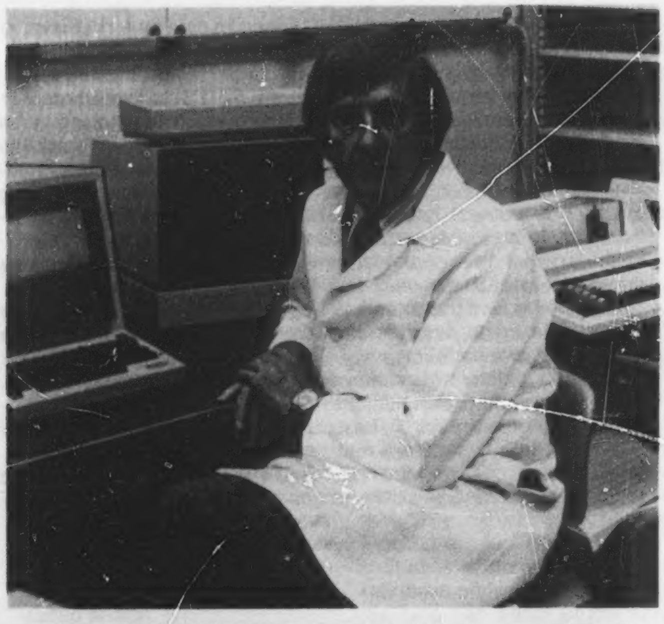
The lung structure of monkeys is much closer to that of humans than to that of rats, says Dr. Last. This difference in structure may account partially for the difference in species sensitivity to ozone. In a recent study, he found that exposure of cynomolgus monkeys to 1.2 ppm of ozone for 1 week resulted in an average eightfold increase in the rate of lung collagen synthesis compared to the threefold increase found in rats, indicating that monkeys may be more sensitive to ozone exposure.

Young monkeys appear to be particularly susceptible to damage from ozone exposure, says Dr. Tyler. "We think that certain levels of ozone exposure might trigger abnormal lung growth. For example, a recently completed study by Dr. Andrew Jackson, Ms. Nancy Tyler, Dr. Last, and myself showed that the lungs of young monkeys grew ab-

Enclosed air pollution chambers: *allowing precise control of ozone concentrations in the air.*



SMOG, *continued*

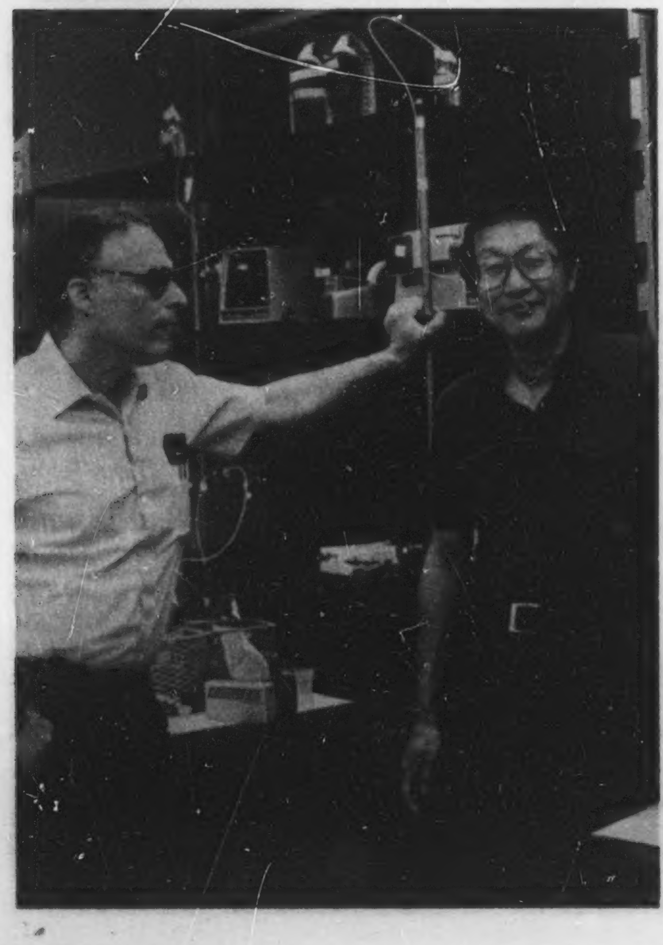


normally when they were exposed to 0.64 ppm of ozone for 8 hours a day for a year." More specifically, the researchers found that 6-month-old cynomolgus monkeys exposed to this ozone concentration for 1 year were less active than an unexposed control group; they developed longer small airways that extended abnormally deep into the lungs; and they accumulated increased lung collagen, indicating increased connective or fibrous tissue in the lung.

Dr. Tyler cautions against extrapolating from these ozone-effects data to conclusions about the effects of ozone on young

Dr. Donald L. Dungworth, director of respiratory research at the California Primate Research Center: *demonstrated that certain levels of ozone caused inflammation of the lung's small airways.*

Dr. Jerold A. Last (left) and Dr. Yuan C. Zee: *demonstrated fibrotic and immunological changes in the lung due to ozone exposure.*



A smoggy day in downtown Los Angeles: *even low levels of ozone in photochemical smog can slightly damage the small airways of the lung.*

Los Angeles Times Photo

children in the Los Angeles Basin, for two reasons: the experimental ozone concentration is higher than the 0.2 ppm concentration that children often encounter on smoggy summer days in Los Angeles, and seasonal changes and children's varied activities do not expose them to consistent ozone levels every day. Dr. Tyler notes, however, that "current air quality standards on ozone are based primarily on experiments involving young adult animals. Children under age 11 are potentially at greater risk because their lungs are still growing and because their playtimes are often in the afternoon during peak ozone levels. We're continuing our study of young monkeys to determine the long-term consequences of chronic exposure to lower ozone concentrations and to assess the precise level of risk."

Adults also vary in their sensitivity to pollutants. For example, people with asthma and other respiratory problems are usually more affected by exposure to ozone than others, says Dr. Yuan C. Zee, professor of microbiology in the department of veterinary microbiology and immunology, University of California at Davis. He, Dr. John W. Osebold, professor of immunology at the university, and their colleagues have conducted several studies showing that even low levels of ozone exposure can cause immunological changes in the lung that worsen allergies and asthma.

Like the rat lung, the mouse lung is structurally different from the human lung. The immune response of the mouse lung, however, is very similar to that of the human lung, according to Dr. Zee. "Immunological mechanisms have been more thoroughly studied in mice than in any other species," he says. "Much that is known about immune responses in man has come from knowledge obtained as a result of mouse studies. In one study we found increased immunological activity in the lung, a clear indicator of tissue injury in mice exposed to 0.4 to 0.64 ppm of ozone for 3 weeks. As far as we know, this is the first definite evidence that lungs damaged by an oxidant air pollutant undergo immunological changes."

Dr. Zee and his colleagues detected an increase in four types of immunoglobulin antibodies—IgM, IgG, IgA, and IgE—in the lungs of mice exposed to ozone. They also found that cells producing IgA, a secretory type of antibody, proliferated along the respiratory membrane surfaces of mouse lungs, indicating membrane damage. This weakening of the membrane allows blood proteins to enter the lung, which in turn causes fluid buildup, making breathing more difficult. With ozone-induced loss of membrane integrity in the lungs, the barrier to antigens—foreign substances that provoke an immune response—might be compromised, exposing the lung to more antigens and perhaps increasing susceptibility to infection, according to Dr. Zee.

He and his colleagues also found that, in mice exposed to 0.64 ppm of ozone for 10 days, cells lining the major airways of the lung lost their capacity to synthesize interferon, which is believed to play an important role in resisting infections. This inhibition of interferon production persisted for a time following return of the mice to unpolluted air. After 10 days in unpolluted air, however, the capacity for interferon synthesis and release reappeared, indicating that repair mechanisms had been at work.

Another immunological study demonstrated that exposure of mice to low levels of ozone enhanced sensitivity to inhaled allergens. Control mice exposed to an inhaled allergen were compared to mice exposed to both the allergen and 0.4 or 0.64 ppm of ozone. Both groups were exposed to aerosolized ovalbumin, a pure protein used to mimic the effects of inhaled flower or plant pollens. Dr. Zee and his colleagues found that an increased number of cells in the lungs of both groups of mice produced IgE antibody, the antigen-specific immunoglobulin involved in allergic and asthmatic responses. The number of IgE-producing cells in mice exposed only to aerosolized ovalbumin increased to almost 9.4 times the normal level. But the number of the IgE-producing cells in lungs of mice exposed to both aerosolized ovalbumin and ozone increased to 34.2 times normal. The reaction of IgE with a specific antigen, in this case aerosolized ovalbumin, evoked a damaging series of reactions leading to bronchial constriction, a reaction that is heightened by ozone exposure.

Dr. Zee believes that this study shows a distinct link between ozone and asthma. "The data suggest that an increase in the total number of asthmatic patients might occur from two effects," he explains. "First, the number of allergically sensitized individuals could rise, as indicated by the results we have reported. Second, after the asthmatic state has become established, it can be predicted that polluted air would irritate the airways of the lung and augment the onset of bronchospasm, thus increasing the number of clinical asthma attacks during high pollution episodes."

Additional reading:

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Research on the inflammatory effects of ozone conducted at the California Primate Research Center was supported by the National Institute of Environmental Health Sciences; the NIH National Heart, Lung, and Blood Institute; and the Animal Resources Program of the NIH Division of Research Resources (DRR). Research on the fibrotic effects of ozone was supported by the National Institute of Environmental Health Sciences; the National Heart, Lung, and Blood Institute; the California Lung Association Research and Medical Education Fund; and the DRR Animal Resources Program. Research on the susceptibility of young monkeys to ozone exposure was supported by the Environmental Protection Agency, the Electric Power and Research Institute, the National Institute of Environmental Health Sciences, and the DRR Animal Resources Program. Research on the immunological effects of ozone was supported by the California Air Resources Board, the DRR Biomedical Research Support Grant Program, and the DRR Animal Resources Program.

Research Focus

Computer System Aids Research Into Visual Field Disorders

A computer system that graphically displays the visual field has been developed to assist researchers in detecting and understanding diseases that impair vision.

According to Dr. William M. Hart, Jr., of the Washington University School of Medicine in St. Louis, the system may allow ophthalmologists to accurately diagnose disorders affecting the retina, optic nerve, and visual pathways of the brain. The research tool, which has been used to test approximately 400 subjects with glaucoma and neurological disorders, including multiple sclerosis, stroke, brain tumors, and encephalitis, could provide diagnoses in early stages of visual disease, thereby allowing prompt treatment to limit damage from the disorder.

The system automates examinations of the visual field, the area seen by the eye at a given moment. It records and processes data into three-dimensional maps that indicate points of restricted sight. These abnormalities, if accurately recorded, may be traced to specific defects in the eye or brain.

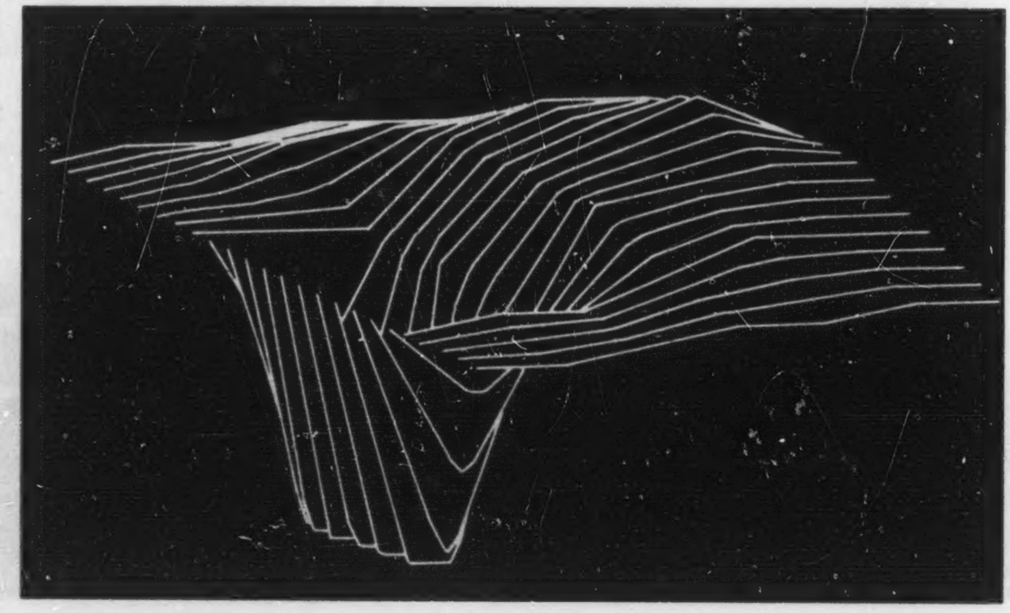
"A major problem of interpreting data from visual field examinations has been that the type of data that can be collected and interpreted has been limited by the inherent complexity of the data itself," says Dr. Hart. "Physicians draw pictures of test results to simplify the recording and storing of data. This restricts the types of examinations to those that provide information that is easily recorded. Because of these limitations, small defects in the visual field sometimes are not detected."

Dr. Hart's research has focused on finding an efficient way of recording, analyzing, and using complex data to produce images that can be easily interpreted. In collaboration with researchers from the Resource for Biomedical Computing at Washington University, he has designed a microprocessor-controlled recording and imaging device that interfaces with the Goldman perimeter, a standard clinical instrument used to test the visual field. In this test, a dot of light is presented to a subject at varying intensities in certain areas of the field of vision. The subject indicates when the light is first perceived. The position and intensity of the dot are recorded and stored on small flexible disks. A computer then processes the data into three-dimensional images.

Dr. Hart says the maps generated from the computer system are only representations of the visual field. "There is no physical relationship between the spatial coordinates of the visual field and the level of visual sensitivity," he says. "The three-dimensional maps are purely conceptual, so there is no intrinsically normal shape to the visual field."

The map of the visual field resembles a mountain with a central peak that corresponds to the point of greatest visual acuity. Visual sensitivity decreases in all directions from this point. Abnormalities in the visual field are depressions in the map that vary in size, shape, and location. The normal blind spot appears as a hole dug into the side of a moun-

Computer drawing of normal visual field: *low mountain with peak (right) represents point of maximum visual acuity. Blind spot is shown as depression below (left).*



tain, says Dr. Hart.

Ophthalmologists render diagnoses by interpreting these maps. The computer system further aids the physician by providing statistical analyses of data obtained during eye examinations and by allowing the retrieval of patient records.

According to Dr. Hart, the system is one of a few of its kind, although several commercial companies are currently constructing other systems with similar capabilities.

The researchers are working to improve the computer's speed and to increase its specificity. This would enable them to differentiate more accurately between diseases that have similar map patterns. Dr. Hart also hopes to study how the visual field changes over time. He says the data might increase understanding of disease development and thereby lead to improved treatment.

"The system promises to be a fundamental advance in the technique of visual field examinations, allowing a more detailed study of specific forms of visual field defects with which we can improve our understanding of the pathophysiological events that lead to disease," says Dr. Hart.

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Dr. Hart's research is supported by the National Eye Institute and the Biotechnology Resources Program of the NIH Division of Research Resources.

Preventing Premature Labor

Slightly more than 9 percent of the estimated 3.5 million babies born in the United States last year were born 3 weeks or more prior to term, according to the National Center for Health Statistics. Premature babies often have serious lung problems, brain hemorrhage, and other disorders.

Currently, beta-adrenergic drugs are given to the mother to prevent early onset of labor by causing the uterine smooth muscle to relax. Many women, however, do not respond to these drugs. If physicians knew what caused labor to begin, they might be able to predict which women are at risk for premature labor and to prevent early labor onset in more patients.

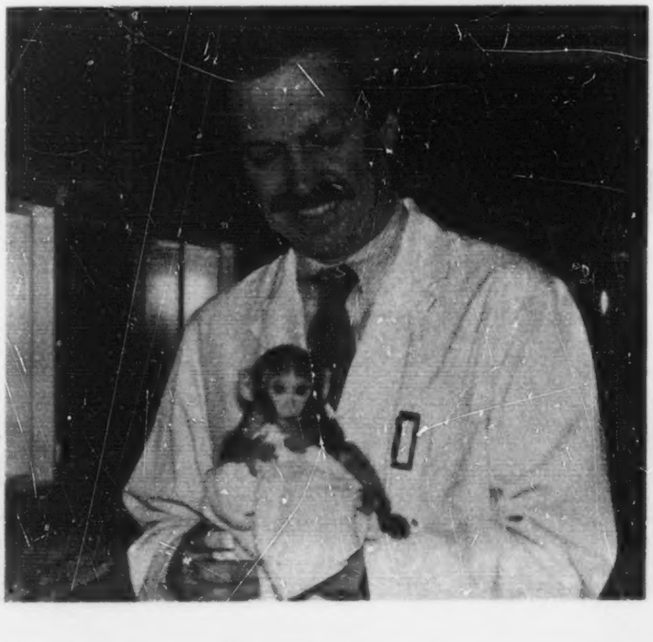
Dr. Miles Novy, a research scientist and practicing obstetrician, has worked with several investigators at the Oregon Regional Primate Research Center in Beaverton to identify the factors that delay or precipitate labor onset. "The delicate balance between hormones released by the mother, fetus, and placenta seems to determine the length of pregnancy and when labor is initiated," says Dr. Novy. "Diurnal patterns in these hormones seem to interact with diurnal rhythms in uterine activity in a way we don't yet completely understand."

Dr. Novy, Dr. Gene Mead, and their colleagues investigated hormonal changes involved in labor onset by inserting capsules of estradiol and progesterone or a blank control capsule into the amniotic fluid of pregnant rhesus monkeys at 125 to 132 days gestation. The animals' normal term is 167 days. They documented that elevated levels of estrone and estradiol, two types of estrogens, in the amniotic fluid predispose the animals to premature delivery and are associated with a significant increase in amniotic fluid prostaglandins prior to vaginal delivery. Prostaglandins are long-chain hydroxy fatty acids that stimulate contractility of the uterine smooth muscle and soften and dilate the cervix before and during labor.

The estrogen surge, which has long been associated with the onset of labor, may result in part from fetal adrenal activity, says Dr. Novy. His research indicates that the level of estrogens in amniotic fluid increases as a result of estrogen precursors produced by the fetal adrenal gland. The placenta transforms these precursors into estrogens, which it then releases into the amniotic fluid.

A rapid rise in amniotic fluid prostaglandins usually follows this estrogen surge and precedes labor onset, according to Dr. Novy. "The current interpretation of how estrogens act to initiate labor," says Dr. Novy, "is that estrogens derived from fetal adrenal precursors stimulate the synthesis of prostaglandins by the outer fetal membranes or the uterine lining, resulting in elevated prostaglandin levels in the amniotic fluid. We have reproduced this effect in monkeys, inducing premature delivery by inserting estrogen capsules into the amniotic fluid."

While data from this study on the role of estrogen are convincing, the progesterone data are inconclusive, says Dr. Novy. Other research with nonprimate species indicates that progesterone relaxes the uterus and that a drop in progesterone levels occurs prior to vaginal delivery. Dr. Novy's group, however, did not find a predelivery drop in progesterone levels. He theorizes that progesterone production in monkeys and other primates may be inhibited by estrogen at the cellular level of the fetal membrane, making it more difficult to measure progesterone withdrawal in the circulation.



Dr. Miles Novy and infant rhesus monkey: *"The delicate balance between hormones released by the mother, fetus, and placenta seems to determine the length of pregnancy and when labor is initiated."*

In earlier research, Dr. Novy had hypothesized that if estrogens played a regulatory role in initiating labor in primates, then suppression of estrogen biosynthesis should prolong gestation. To test the hypothesis, he, Dr. Scott Walsh, and Mr. Michael Cook administered dexamethasone, a potent synthetic glucocorticoid that suppresses fetal and maternal adrenal activity and thus estrogen biosynthesis, to 14 pregnant rhesus monkeys from day 130 of gestation until delivery. They found that dexamethasone decreased basal levels of maternal estradiol and cortisol, but not progesterone, and abolished the prepartum estrogen surge. Decreased estrogen biosynthesis resulted in prolonged gestation, with 71 percent of the fetuses born more than 8 days past term. This same study showed that dexamethasone did not precipitate labor as glucocorticoids are known to do in sheep, a species often used in obstetrical research. "If anything, glucocorticoids tend to delay labor in primates and probably augment labor-preventing beta-adrenergic therapy," asserts Dr. Novy.

This information is important to obstetricians treating women who show signs of premature labor at 29 or 30 weeks into pregnancy, explains Dr. Novy. The usual clinical procedure is to give them a beta-adrenergic drug such as ritodrine intravenously for 12 to 24 hours to relax the uterus and a glucocorticoid such as dexamethasone for 24 to 48 hours to accelerate development of the fetal lungs, which are among the last fetal organs to mature. The beta-adrenergic drug is then administered orally or subcutaneously to delay labor onset and to enable the fetus to mature sufficiently to avoid or minimize the health risks of premature birth.

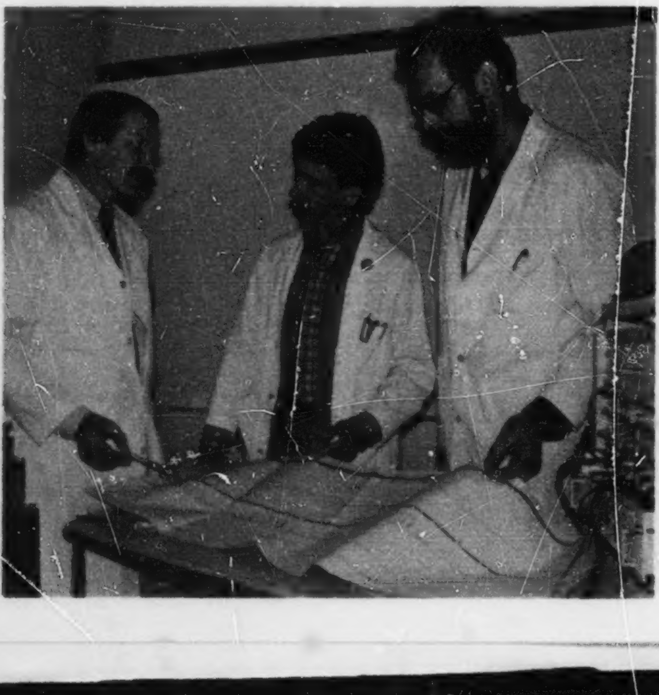
If given in moderate doses, beta-adrenergic drugs do not appear to have adverse effects on the fetus and cause only minor problems such as tachycardia in the mother, says Dr. Novy. Stronger doses and prolonged treatment, however, increase the chances of pulmonary edema, serious cardiovascular problems, and altered carbohydrate metabolism in the mother. "Our study indicates that during the 24- to 48-hour period that dexamethasone is used for fetal lung development, it is probably safe for mother and fetus," says Dr. Novy. "The same study showed, however, that when larger doses of dexamethasone are administered for longer periods of time, the incidence of stillbirth increases."

Dr. Novy says the exact mechanism by which dexamethasone delays labor onset in rhesus monkeys is not known, but he offers several possible explanations. Dexamethasone might inhibit the fetal adrenal gland from producing estrogen precursors, thus preventing the surge in amniotic fluid estrogens associated with labor onset, or the drug may inhibit the production of arachidonic acid needed for synthesis of prostaglandins. Since glucocorticoids increase the number or affinity of beta-adrenergic receptors in some tissues, it is also possible that dexamethasone delays parturition by a direct effect on the binding of beta agonists to receptors in the uterus. Increased binding would relax the uterus.

Having demonstrated that dexamethasone delays labor in primates, Dr. Novy began to investigate its effect on uterine activity. In a study recently completed, Dr. Charles Ducsay, Dr. Novy, and their colleagues demonstrated that dexamethasone induces changes in the 24-hour rhythm of uterine activity, a rhythm they were among the first to quantify, says Dr. Novy. To determine uterine activity patterns, the investigators placed amniotic pressure catheters in five rhesus monkeys, one each on days 127 to 131 of gestation, and kept them in place until delivery. During each 24-hour period, the animals were exposed to 16 hours of

Rhesus monkey wearing nylon suit and telemetry backpack for continuous recording of amniotic fluid pressure: *recording uterine activity documented a definite uterine circadian pattern with peak uterine activity occurring between 11 p.m. and 2 a.m.*

Dr. Miles Novy (left), Dr. Charles Ducsay, and Mr. Michael Cook (right): *examining patterns of uterine activity from catheterized pregnant rhesus monkeys.*



light and 8 hours of darkness, corresponding to usual human waking and sleeping times. By continuously recording uterine activity, the researchers observed a definite circadian pattern, with peak uterine activity occurring during the dark period between 11 p.m. and 2 a.m. "It has been shown that there is probably a similar uterine activity pattern in women, but researchers haven't yet quantified the contractions," elaborates Dr. Novy. "Clinically we observe that labor onset usually starts in the late evening, with delivery occurring 24 to 36 hours later."

To examine dexamethasone's effects on uterine activity rhythms, the researchers infused the drug via maternal venous catheter in four pregnant rhesus monkeys following a 48-hour control period. They found that dexamethasone initially reduced mean uterine activity and then eliminated the nocturnal peak. Although mean uterine activity returned to preinfusion levels 48 hours following dexamethasone infusion, the nocturnal peak was still eliminated.

"We still don't know what's driving the uterine circadian rhythms," says Dr. Novy, "whether it's the maternal autonomic nervous system, the light-dark cycle, or both of these in conjunction with some fetal events." Dr. Novy and his colleagues are currently correlating changes in uterine activity with changes in maternal, fetal, and amniotic fluid hormone levels and relating these data to labor onset. "Preliminary data from the ongoing study suggest that uterine activity patterns are synchronized with diurnal rhythms in the secretion of fetal steroids," says Dr. Novy. "It seems that the fetal adrenal gland becomes active at night, producing more products such as estrogen precursors at the same time that uterine activity increases.

"We know that, at night, increased activity of the fetal adrenal gland, indicated by levels of estrogen precursors such as dihydroepiandrosterone sulfate (DHEAS), corresponds with peak uterine activity," he adds. "The fluctuation in DHEAS indicates a fetal rhythm, but it may be a passive one that responds to changes in maternal adrenal activity. Even though increased fetal adrenal activity at night coincides with peak uterine activity, a cause and effect relationship is far from established. What doesn't fit is that maternal estrogens peak in the early morning and maternal progesterone levels increase at night. We would expect increased estrogen levels and lowered progesterone levels with increased uterine activity."

High maternal progesterone levels in the evening are probably due to the competition between progesterone and cortisol for a protein in the plasma called cortisol-binding globulin (CBG), says Dr. Novy. "In the morning, when the maternal adrenal gland is producing a lot of cortisol, there is less progesterone bound to the protein, so we measure less progesterone. But at night the maternal adrenal gland produces less cortisol, so there are more CBG receptors available to bind to progesterone. We're measuring more progesterone because progesterone clearance is reduced. Since more of the available progesterone is bound to CBG, there may be a decrease in free progesterone, which some researchers believe is its biologically active form. Therefore, levels of biologically active progesterone may not really be elevated at the same time as increased uterine activity. But all of these data need to be sorted out. That's what we're trying to do now.

"We know that labor is initiated by a combination of maternal, fetal, and uterine events that selectively interact with one another," he adds. "It is possible that once these events are isolated and their interaction pinpointed, one could predict which patients are at risk for premature labor and take the necessary precautions, or one could design a safe and effective drug as an alternative for women threatened with premature labor who do not respond to currently available beta-adrenergic drugs."

Dr. Miles Novy implanting intrauterine fetal catheter in pregnant rhesus monkey: *the catheter enables researchers to monitor uterine activity.*

Additional reading:

1. Novy, M. J., Endocrine and pharmacological factors which influence the onset of labor in rhesus monkeys. In *The Fetus and Birth* (O'Connor, M., and Knight, J., eds.), Ciba Foundation Symposium. Amsterdam and New York, Elsevier/Excerpta Medica, 1977, pp. 259-295.
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3. Ducsay, C. A., Cook, M. J., Walsh, S. W., and Novy, M. J., Circadian patterns and dexamethasone-induced changes in uterine activity in pregnant rhesus monkeys. Presented at the 29th Annual Meeting of the Society for Gynecologic Investigation, Dallas, Tex., Mar., 1982.
4. Novy, M. J., Walsh, S. W., and McCarthy, M. S., Dexamethasone delays parturition in primates. Presented at the 27th Annual Meeting of the Society for Gynecologic Investigation, Denver, Colo., Mar., 1980.
5. Novy, M. J., Walsh, S. W., and Cook, M. J., Chronic implantation of catheters and electrodes in pregnant nonhuman primates. In *Animal Models in Fetal Medicine*, (Nathanielsz, P., ed.). Amsterdam and New York, Elsevier/Excerpta Medica, 1980, pp. 133-168.

Research on the factors involved in primate parturition is supported by the March of Dimes Birth Defects Foundation, the Animal Resources Program (ARP) of the NIH Division of Research Resources, and the National Institute of Child Health and Human Development. The Oregon Regional Primate Research Center is funded by the ARP.

Research Resources Reporter
August 1982

Information Exchange

Research Highlights

A live cytomegalovirus (CMV) vaccine has shown early promise in a trial now under way with normal volunteers and kidney transplant candidates at the University of Pennsylvania School of Medicine in Philadelphia.

CMV can cause severe congenital disease. One in every 1,000 babies born in the United States will have some clinical signs or symptoms of CMV infection at birth or during the early years of life, according to estimates by the Centers for Disease Control in Atlanta. Every year between 3,000 and 6,000 infants are profoundly affected by CMV; that is, they are either mentally retarded or have some other serious defect, according to estimates by the National Institute of Allergy and Infectious Diseases.

Adults may also develop serious CMV infections. Those who are most susceptible have malignancies or immunologic defects or are undergoing immunosuppressive therapy. Other groups at risk include blood transfusion recipients and nurses. Donated blood may contain CMV. Nurses who work in pediatric hospitals are at increased risk of infection through contact with infected infants. Surveys indicate that 50 percent or more of individuals age 20 to 29 in the United States do not have demonstrable antibodies to CMV.

In the vaccine trial in Philadelphia, being conducted by Dr. Stuart E. Starr and his colleagues, all normal volunteers demonstrated both cellular and humoral immune responses to the live Towne strain of CMV vaccine. Five of the eight immunized renal transplant candidates showed lymphocyte proliferation responses comparable to those of normal controls. In the normal volunteers, antibodies and cellular responses appeared early after vaccination. Antibody titers of more than 1:1,000 and high lymphocyte proliferation counts were found within 2 to 3 weeks after injection of the vaccine. "Both cellular and humoral immunity persisted for at least 10 months after vaccination of normal adults," Dr. Starr reports.

Immune responses were delayed, however, in the kidney transplant candidates and in some instances did not peak until 12 weeks after vaccination. Dr. Starr emphasizes that six of these patients were undergoing hemodialysis therapy. Research has demonstrated that factors in the blood of some dialysis patients suppress lymphocyte proliferation. The renal transplant patients in the present study were not immunosuppressed by medication, but Dr. Starr says that, due either to their underlying kidney disease or to the hemodialysis itself, hemodialysis patients are not immunologically normal.

The attentuated Towne CMV vaccine used in the present study was developed by Dr. Stanley A. Plotkin of the Children's Hospital of Philadelphia, who is also the senior member of the research team. Extensive testing proved the vaccine to be safe for clinical efficacy trials.

Studies with strains of CMV other than the vaccine strain showed that lymphocytes from vaccinated subjects "could recognize antigens in other strains of the virus," says Dr. Starr. "Such cross-reactivity should enhance the possibility that the vaccine will afford some degree of protection against natural infection."

He points out that the real test of the vaccine's effectiveness in the current trial will be whether it protects the kidney transplant candidates *after* the transplantation. In one survey, donor kidneys seemed to be the source of CMV infection in 83 percent of the seronegative recipients.

Additional reading:
1. Starr, S. E., Glazer, J. P., Friedman, H. M., Farquhar, J. D., and Plotkin, S. A., Specific cellular and humoral immunity after immunization with live Towne strain cytomegalovirus vaccine. *Journal of Infectious Diseases* 143: 585-589, 1981.
2. Starr, S. E., Cytomegalovirus. *Pediatric Clinics of North America* 26:283-293, 1979.

Studies with the live Towne strain cytomegalovirus vaccine are supported in part by the Biomedical Research Support Grant Program of the NIH Division of Research Resources and the Hassel Foundation.

An improved system for measuring the effect of bacteria on respiratory tract cilia was developed recently by researchers at the University of Tennessee in Knoxville.

Drs. David A. Bemis and John R. Kennedy of the microbiology and the zoology departments respectively used ciliated epithelial cell outgrowths from canine tracheal tissue to study the ciliostatic action of *Bordetella bronchiseptica*, the causative agent of bronchopneumonia in dogs. Using this system, the researchers detected significant reduction in the motion of respiratory tract cilia within 5 minutes after introducing the bacteria into cell culture. Ciliary activity had nearly stopped 3 hours later. The earliest detected changes in ciliary activity previously reported with the use of other measurement systems occurred only after 12 hours.

Ciliated outgrowths were prepared from explants of canine tracheal mucosa removed at necropsy from healthy adult mixed-breed dogs. After 2 to 10 days of culturing the cells, outgrowths were four to six times larger than the original explant and contained about 20 percent ciliated cells.

The effect of bacteria on the cilia was observed by phase-contrast microscopy in conjunction with a television camera and monitor. The frequency of ciliary beat was measured in sequence by a photomultiplier, an oscilloscope, and a spectrum analyzer. A scanning electron microscope was used to study the attachment of bacteria to cilia.

Respiratory tract cilia in mammals clear bacteria and other foreign material from the tract. Rhythmic beating of the cilia sweeps the mucous layer with entrapped debris upward for expulsion. In *Bordetella* infections such as whooping cough and bronchopneumonia, however, the bacteria adhere and grow among the cilia, gradually stopping their action. The diphtheria organism as well as *Hemophilus influenzae* and *Mycoplasma pneumoniae* also inhibit the action of cilia in the respiratory tract.

Examination of the bacteria-treated outgrowths with the scanning electron microscope revealed attachment of virulent organisms to cilia in large numbers, causing the cilia to stick together. The researchers noted that the bacteria attached mainly to the upper half of the cilia. Multiple attachment of a bacterium to a given cilium and attachment of the same organism to more than one cilium were also observed.

The action of untreated cilia in the test system was fairly constant at 8.5 beats per second. The researchers found that addition of virulent phase I *B. bronchiseptica* significantly reduced beating of the cilia within 5 minutes. After half an hour the frequency was reduced by 55 percent; at the end of 3 hours cilia in 80 to 90 percent of the cells ceased beating completely. Cilia in the remainder of the cells were moving at less than 1 beat per second. A less virulent strain as well as heat-killed and formalin-killed cultures of the virulent strain had no effect on ciliary activity. The researchers found, however, that an attenuated live vaccine strain caused a reduction in ciliary activity.

The researchers point out that although the ciliostatic activity of virulent *B. bronchiseptica* is probably involved in causing disease, it cannot be solely responsible, since the attenuated vaccine strain also reduces ciliary activity.

In an effort to determine the factor or factors responsible for the ciliostatic effect of *B. bronchiseptica*, the researchers tested both endotoxin from the bacterium and a filtrate from a culture of the bacterium. Neither product had any adverse effect on ciliary activity.

These tests were prompted by research showing that gonococcal endotoxin inhibits the activity of cilia in the human oviduct and also that exotoxins of whooping cough and diphtheria organisms, as well as those from several other pathogens, inhibit the action of cilia in the human respiratory tract.

The investigators concluded that further research is necessary to determine the factor or factors responsible for the ciliostatic activity of *B. bronchiseptica*.

Additional reading:

1. Bemis, D. A., and Kennedy, J. R., An improved system for studying the effect of *Bordetella bronchiseptica* on the ciliary activity of canine tracheal epithelial cells. *Journal of Infectious Diseases* 144:349-357, 1981.
2. Bemis, D. A., Greisen, H. A., and Appel, M. J. G., Pathogenesis of canine bordetellosis. *Journal of Infectious Diseases* 135:753-762, 1977.

Research on the effect of Bordetella bronchiseptica *on canine ciliated tracheal epithelial cells was supported by the Biomedical Research Support Grant Program of the NIH Division of Research Resources, the Cystic Fibrosis Foundation, and the Wellcome Research Laboratories.*

Researchers at Bishop College in Dallas are using a virus that attacks certain plants as a model of viral infection.

"We hope that by studying brome mosaic virus (BMV), we may be able to understand more clearly how viral genes interact in causing disease and to apply this knowledge to viruses that attack higher organisms," says Dr. Edison R. Fowlks, professor and chairman of the Bishop life sciences department. Specifically, he and his associates are trying to understand the processes of viral RNA replication and translation in relation to gene structure and function.

They are using BMV as a model because its replication and protein assembly processes involve only a few genes and because its genetic material is structurally similar to that of many medically important viruses. Like the viruses that cause influenza, polio, and rubella, BMV's genetic material is distributed among several molecules of RNA. In nature genetic recombination between strains of BMV may produce a hybrid with a different disease-causing capability, according to Dr. Fowlks.

In 1970 Dr. Fowlks and Dr. Ronald Young of the University of California at Berkeley discovered that the genetic material of the BMV is distributed among particles of similar molecular weight. RNA's 1 and 2 are contained in independent particles and the other two molecules, RNA's 3 and 4, are contained within the same particle.

In a recent study, the researchers discovered a unique nucleotide sequence in RNA 3 that they believe may regulate the expression of the coat protein gene and the processing of RNA 4. Dr. Fowlks and Dr. Yuan Fon Lee at Bishop College found that RNA 3 contains a series of repeating adenine nucleotides that are sandwiched between its other nucleotides. These sequences, called poly A, vary in length between 18 and 25 nucleotides and, according to their studies, may be located at the junction of the two genes in RNA 3.

Poly A sequences have been noted in the cells of higher organisms as well as in bacteria, fungi, and viruses. These sequences, however, exist in long stretches of approximately 200 nucleotides and are found only at the end of RNA molecules. Although several hypotheses have been proposed to explain the role of poly A, its function is unknown.

Dr. Fowlks and his colleagues believe that there may be a cor-

Ciliated epithelial cells in outgrowths from canine tracheal explants (left): *Bordetella bronchiseptica attach to cilia (right), clumping the cilia and inhibiting their motion.*

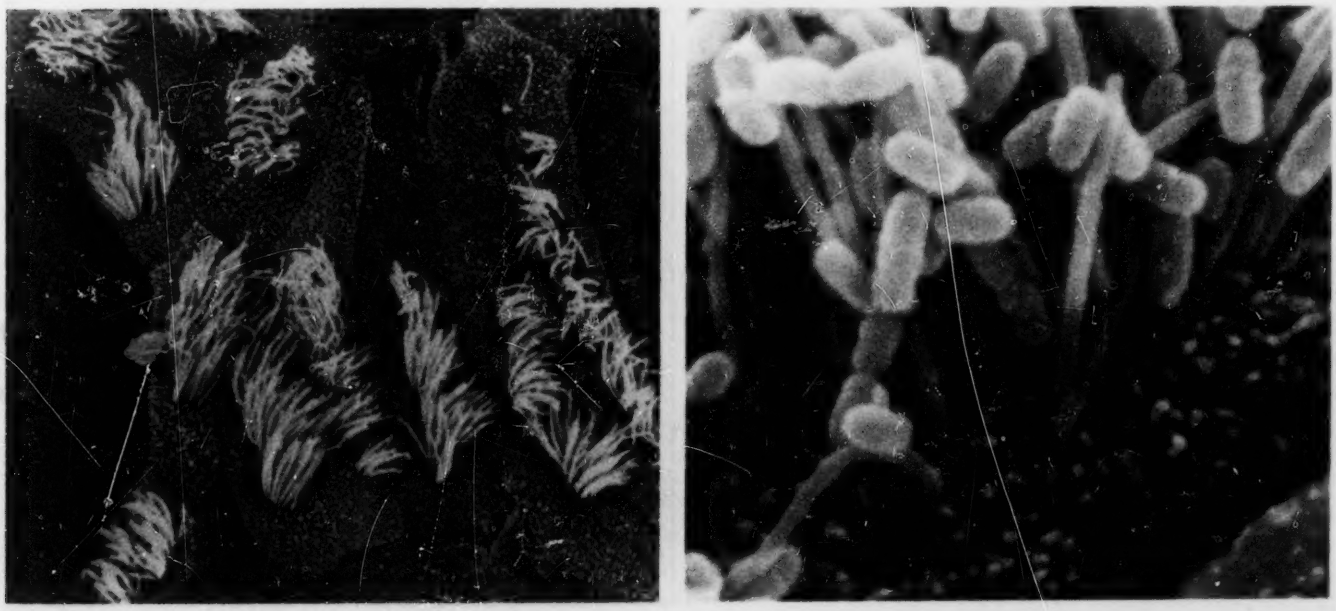

Photo courtesy of *The Journal of Infectious Diseases*, Drs. David A. Bemis and John R. Kennedy © 1981 by the University of Chicago, Publisher

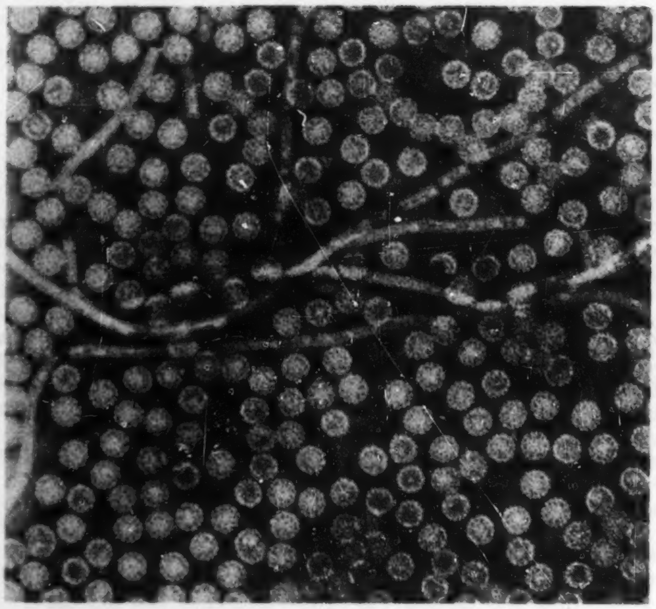

Photo courtesy of Dr. Robley Williams, Univ. of California at Berkeley

Spherical particles of brome mosaic virus: *a model to study viral RNA replication and translation.*

relation between the unique poly A sequence in the RNA 3 molecule of BMV and the expression of the gene that codes for the coat protein, a gene present in both RNA 3 and 4. The coat protein surrounds the nucleic acid of the virus, which invades host cells. It protects the nucleic acid until it reaches its target and participates in the infection of the cell.

The researchers propose that the length of the internal poly A chain may control the production of the coat protein. RNA strands containing short poly A segments may inhibit synthesis of the protein, they believe. RNA strands that do not contain poly A or that contain long poly A segments may provide information for synthesis of the protein.

The researchers' explanation is compatible with other research on messenger RNA translation. Histone messenger RNA, which lacks poly A, can be translated for production of its coat protein in vitro as well as in vivo. Globin RNA, when freed of its externally located poly A, can also be translated in vitro. "These and other studies raise the possibility that the messenger RNA of other systems may contain a short poly A sequence that inhibits translation. This inhibition may be removed partially or completely by poly A elongation or by cleavage of poly A," says Dr. Fowlks.

The research was conducted using a method called "RNA fingerprinting," which allows molecular biologists to analyze gene fragments. The procedure consists of exposing each of the RNA molecules labeled with ^{32}phosphorus to an enzyme that breaks the nucleic acid into small fragments. The fragments are then separated electrically according to molecular weight, using two-dimensional gel electrophoresis. Drs. Fowlks and Lee improved three existing RNA labeling procedures and modified the two-dimensional gel electrophoresis system, which enabled more thorough study of the RNA's in the brome mosaic and other viruses.

Additional reading:
1. Fowlks, E. R., and Lee, Y. F., Detection and sequence of an internal A-rich T_1 oligonucleotide series in brome mosaic viral

"Fingerprint" of RNA in brome mosaic virus: *improved technique reveals that BMV contains a series of adenine-rich RNA fragments (1 to 8).*

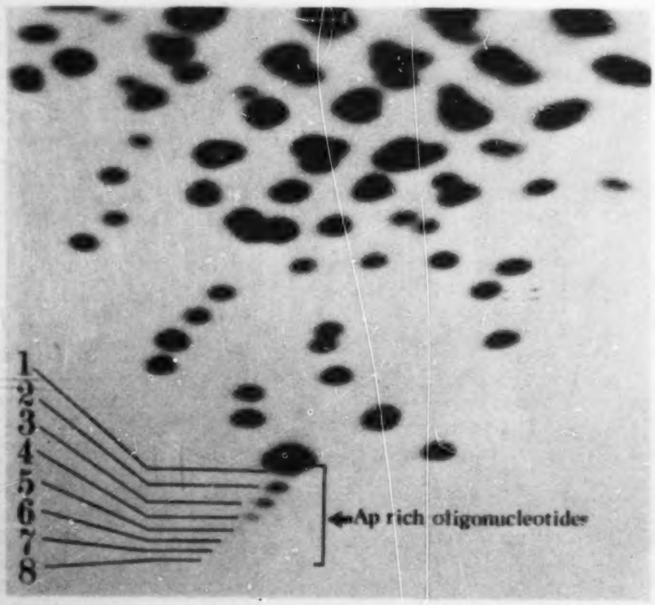

Photo courtesy of Elsevier/North Holland Biomedical Press, printed in FEBS Letters 130:34, 1981.

Nucleotide sequence of adenine-rich spot number 2: *using rapid sequencing gel electrophoresis to confirm the existence of internally located poly A.*

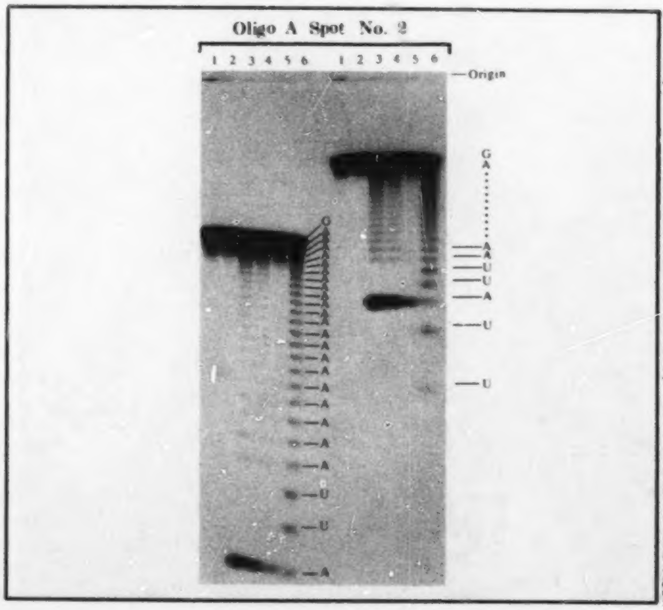

Photo courtesy of Elsevier/North Holland Biomedical Press, printed in FEBS Letters 130:34, 1981

RNA 3. *FEBS Letters* 130:32-38, 1981.
2. Fowlks, E. R., and Young, R. J., Detection of heterogeneity in plant viral RNA by polyacrylamide gel electrophoresis. *Virology* 42: 548-550, 1970.
3. Manning, P., and Fowlks, E. R., Detection of internal poly A in brome mosaic viral RNA. *Eighth Annual Minority Biomedical Support Symposium*, 1980, p. 126.
4. Lee, Y. F., and Fowlks, E. R., Rapid in vitro labeling procedures for two-dimensional gel fingerprinting. *Analytical Biochemistry* 119:224-235, 1982.

Research on poly A is supported by the Minority Biomedical Research Support Program of the NIH Division of Research Resources and by a Research Career Development Award from the National Institute of Allergy and Infectious Diseases.

Research Service

Resources for Brain Tissue

Two brain tissue repositories, one in Los Angeles, the other near Boston, are providing normal and diseased brain tissue, cerebrospinal fluid, and peripheral tissue such as selected muscle samples to researchers and clinicians working in neurology and psychiatry. Samples are suitable for biochemical and structural studies in pathology and for research into special diagnostic problems.

The National Neurological Research Bank, located at the Veterans Administration's Wadsworth Medical Center in Los Angeles, California, provides specimens taken during autopsy from patients who had multiple sclerosis, Huntington's disease, epilepsy, Parkinson's disease, amyotrophic lateral sclerosis, hereditary ataxia, dementia, Alzheimer's disease, senile dementia, subacute sclerosing panencephalitis, Tourette's disease, and mental retardation. Tissues from patients who displayed schizophrenia, manic-depressive psychosis, and drug or alcohol abuse are stocked at the resource, as are tissues from donors who committed suicide.

The Brain Tissue Resource Center, located at McLean Hospital in Belmont, Massachusetts, primarily handles tissues from patients who had Huntington's disease, Parkinson's disease, Alzheimer's disease, schizophrenia, Tourette's disease, and dystonia.

Both resources also supply normal brain tissue for biomedical and structural comparisons with diseased samples.

Specimens range from whole brains to specific tissue, fluid, and cells. Patient histories can be detailed, including age, sex, race, and socioeconomic factors, or they can be very general. The determining factor in all cases is the research need of the investigator.

Advice on conducting the research is given by resource scientists. Specimens and advice are cost free for projects approved by Dr. Wallace W. Tourtellotte and his staff in Los Angeles or by Dr. Edward D. Bird and his colleagues in Belmont.

The resources allow investigations that bridge the gap between medical and animal studies of neurological diseases. Animal models can only approximate human pathology.

Necessarily, much of the research directed at controlling or preventing neurological diseases must be done on human tissue. A few large medical institutions stock brain tissue for limited in-house studies, but most do not have the needed facilities to supply extramural investigators.

By supporting the two repositories, the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the National Institute of Mental Health (NIMH) are attempting to centralize the supply of brain tissue. The two national centers, both funded partially by NINCDS and NIMH, are the American components of an international network of brain tissue repositories. Representatives from centers around the world meet periodically, usually every 18 months, to discuss procedures for collection, storage, dissection, and dissemination of samples and to standardize definitions of brain areas.

Standards for the time between patient death and the freezing of tissue, the temperatures at which tissues are frozen, and how one donor's tissue can be prevented from contaminating tissue from other donors are among the

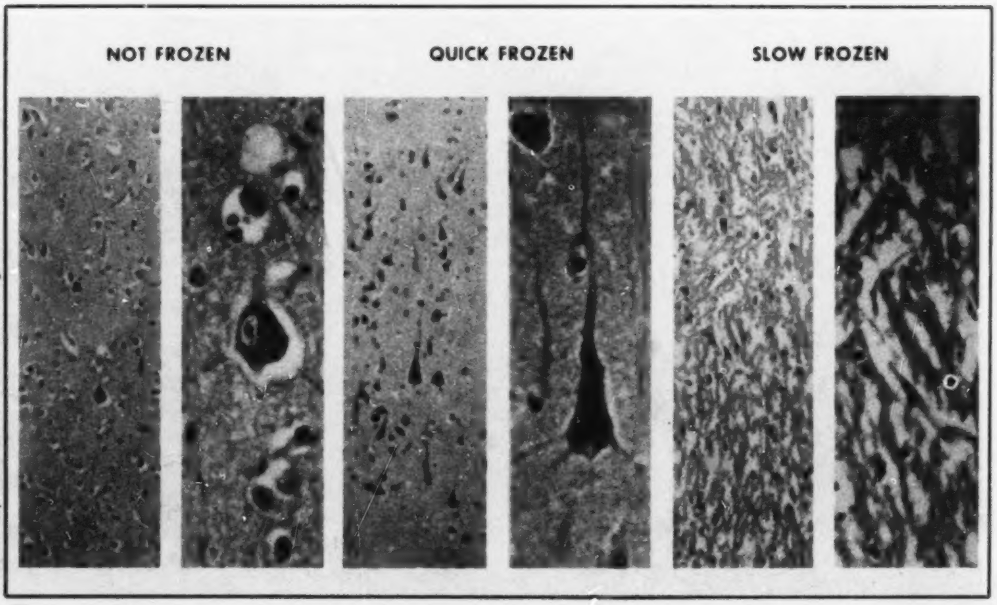

Photo courtesy of the National Institute of Neurological and Communicative Disorders and Stroke

The storage of tissue at brain repositories: *providing specimens for neurological research.*

issues decided by consensus. There has also been agreement on handling and shipping procedures to ensure that tissue is not damaged before analysis. These and other procedures are continually modified to meet the needs of the majority of neurological investigators served by the respective centers.

Conferences have focused on setting boundaries of brain substructures such as the nucleus accumbens, which has been tentatively associated with schizophrenia, and the nucleus basalis, which has been shown to degenerate in patients with Alzheimer's disease. According to Dr. Thomas Chase, NINCDS project officer of the U.S. resources, textbooks in neuroanatomy have typically ignored or ambiguously mentioned these substructures because, until recently, they appeared to have little medical significance. Although a controversy still persists concerning their significance and actual dimensions, the establishment of standardized boundaries for these and other subsections of the brain exactly defines what tissues are being examined. Such standards are particularly important, says Dr Chase, because most clinical researchers in the neurosciences are highly specialized and may have difficulty differentiating tissues that have distinctly different functions but very similar appearance. Standards employed by the two centers reduce the risk of conflicting results obtained by various research groups.

Tissue supplies depend largely on the interests of the scientific community. An unexpected finding in one area of research may dramatically increase the demand for a specific type of tissue. When a large number of investigators converge on the same subject, tissue stocks are reduced, causing delays in responding to requests.

Physicians and investigators who need brain tissue samples or advice in conducting analyses of brain tissue should contact the national resources directly. Information can be obtained by writing to or calling:

W. W. Tourtellotte, M.D., Ph.D.
Director
The National Neurological Research Bank
VA Wadsworth Medical Center/127
Los Angeles, California 90073
Telephone (213) 824-4307 or 478-3711, ext. 41771, or FTS 794-4171

or

Edward D. Bird, M.D.
Director
The Brain Tissue Resource Center
McLean Hospital
Belmont, Massachusetts 02178
Telephone (617) 855-2400

Questions and Answers

Q

Where can one obtain chinchillas for auditory research purposes?

Laboratory of Auditory Research
Department of Biology
University of Pennsylvania
Philadelphia, Pennsylvania
19104

A

The following chinchilla breeders in your state can probably supply the animals needed for your research:

Oakwood Acres Chinchilla Farm
Route 19
Harmony, Pennsylvania 16037
Telephone (412) 368-8306

Thompson Chinchilla Ranch
639 Washington Street
Royersford, Pennsylvania 19468
Telephone (215) 948-3672

Q

I would appreciate information on the availability of African green monkeys.

Dr. O. J. Oyen
School of Dentistry
Case Western Reserve University
Cleveland, Ohio 44106

A

African green or vervet or grivet monkeys (*Cercopithecus aethiops*) can be obtained from:

Matthew Block
Worldwide Primates, Inc.
7780 Northwest 53rd Street
Miami, Florida 33166
Telephone (305) 885-7834

Thomas Ferrell
Hazleton Research Primates
1946 Isaac Newton Square, West
Reston, Virginia 22090
Telephone (703) 471-4646

Michael A. Nolan
Charles River Research Primates Corporation
P.O. Box 416
Port Washington, New York 11050
(516) 883-1800

Chinchillas (*Chinchilla laniger*): subjects for auditory research.

Reporter Index Now Available

A cumulative index of the first four volumes of the *Research Resources Reporter* has been published and is now available.

The index follows MeSH, the medical subject headings system of the National Library of Medicine. It covers the years 1977 through 1980.

A single copy of the *Reporter* index may be obtained free of charge by writing to the Research Resources Information Center, 1776 East Jefferson Street, Rockville, Maryland 20852 or to the Office of Science and Health Reports, Division of Research Resources, National Institutes of Health, Bethesda, Maryland 20205.

Standards Published for Laboratory Tests

Two publications, one describing methodology for laboratory tests concerning anaerobic bacteria and the other describing collection, transport, and testing of blood specimens, are available from the National Committee for Clinical Laboratory Standards (NCCLS).

Tentative Standard Reference Agar Dilution for Antimicrobial Susceptibility Testing of Anaerobic Bacteria explains M11-T, a reference method that standardizes procedures for the preparation of inocula, antibiotics, test plates, inoculation, and interpretations for dilution susceptibility testing of anaerobic bacteria.

The subcommittee on susceptibility testing of anaerobic bacteria, chaired by Dr. Ronald J. Zabransky of the Mount Sinai Medical Center, recommends the use of M11-T to ensure that possible variations are not introduced by individual laboratories when testing anaerobic bacteria for susceptibility to antimicrobials.

Tentative Guidelines for the Standardized Collection, Transport, and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays explains H21-T, a set of general procedures to be employed when performing coagulation assays. According to a representative of NCCLS, these guidelines will help eliminate many of the variables that may affect test results. They are intended to aid laboratory and clinical personnel responsible for obtaining patient specimens, performing coagulation tests, and controlling the quality of these tests.

The subcommittee on coagulation, chaired by Dr. Harvey R. Gralnick of the National Institutes of Health, is currently working on subsequent guidelines that will deal specifically with prothrombin time, activated partial thromboplastin time, fibrinogen concentration, and coagulation factors.

Each publication sells for $9 plus $1 for orders placed outside the United States. Requests with accompanying check or money order should be addressed to: The National Committee for Clinical Laboratory Standards, 771 East Lancaster Avenue, Villanova, Pennsylvania 19085.

Meetings

International Symposium On Blood Substitutes

The 1982 International Symposium on Blood Substitutes will be held September 29 to October 1, 1982, in San Francisco, California. Discussion of blood substitutes for clinical, civilian, and military applications will be of particular interest to scientists, surgeons, pharmaceutical representatives, and military physicians. The symposium will cover current research programs and will emphasize clinical and logistical considerations for blood substitutes, specifically the application of perfluoro-chemicals and stroma-free hemoglobin solutions.

Sponsored by the Letterman Army Institute of Research, U.S. Army Medical Research and Development Command, and the National Institutes of Health, the symposium is being coordinated by Battelle's Columbus Laboratories. For more information contact:

Denise Cooley
Battelle's Columbus
Laboratories
505 King Avenue
Columbus, Ohio 43201
Telephone (614) 424-5532

August 22-26, 1982

Title: *6th Congress of the International Association for the Scientific Study of Mental Deficiency*
Location: *Toronto, Canada*
Contact: *Professor G. A. Roeher*
National Institute on Mental Retardation
York University Campus
4700 Keele Street
Downsview, Ontario M3J 1P3
Canada

August 23-27, 1982

Title: *9th International Congress of Transplantation*
Location: *Brighton, England*
Contact: *Professor L. Brent*
Department of Immunology
St. Mary's Hospital Medical School
Norfolk Place
London W2 1PG, England

September 10-11, 1982

Title: *4th Neonatal/Perinatal Fall Symposium: Pharmacology and Therapeutics*
Location: *Amherst, New York*
Contact: *Rayna Dutton*
Coordinator
Continuing Medical Education
Children's Hospital
219 Bryant Street
Buffalo, New York 14222
Telephone (716) 878-7630

September 13-15, 1982

Title: *Nutrition and Blood Pressure Control*
Location: *Arlington, Virginia*
Contact: *International Life Sciences Institute*
Suite 600
900 17th Street, N.W.
Washington, D.C. 20006
Telephone (202) 659-0074

September 14-17, 1982

Title: *International Vascular Symposium*
Location: *London, England*
Contact: *Symposium Secretariat*
Conference Associates IVS
34 Stanford Road
London W8 5PZ, England

September 22-24, 1982

Title: *Histopathology Seminar on the Respiratory System of Laboratory Animals*
Location: *Hanover, Federal Republic of Germany*
Contact: *International Life Sciences Institute*
Suite 600
900 17th St., N.W.,
Washington, D.C. 20006
Telephone (202) 659-0074

September 29-October 2, 1982

Title: *Annual Meeting of the American Society of Human Genetics*
Location: *Detroit, Michigan*
Contact: *Dr. Lester Weiss*
Genetics
CFP-446
Henry Ford Hospital
2799 West Grand Boulevard
Detroit, Michigan 48202
Telephone (313) 876-3116

September 29-October 2, 1982

Title: *Annual Meeting of the American Neurological Association*
Location: *Washington, D.C.*
Contact: *J. F. Toole, M.D.*
Department of Neurology
Bowman Gray School of Medicine

Winston-Salem, North Carolina
27103
Telephone (919) 748-2336

October 3-8, 1982

Title: *33rd Annual Session of the American Association for Laboratory Animal Science*
Location: *Washington, D.C.*
Contact: *Joseph Garvey*
Suite 205
210 North Hemmes Avenue
Joliet, Illinois 60435
Telephone (815) 729-1161

October 5-7, 1982

Title: *Cellular Systems for Toxicity Testing*
Location: *New York City, New York*
Contact: *Conference Department*
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021
Telephone (212) 838-0230

October 15-17, 1982

Title: *International Meeting on Fetoscopy Uses for Prenatal Diagnosis and In Utero Therapy*
Location: *Tiburon, California*
Contact: *Mitchell S. Golbus, M.D.*
Department of Obstetrics, Gynecology, and Reproductive Sciences
University of California, San Francisco
San Francisco, California 94143
Telephone (415) 666-4157

October 16-21, 1982

Title: *7th International Symposium of the World Association of Veterinary Microbiologists, Immunobiologists, and Specialists in Infectious Diseases*
Location: *Barcelona, Spain*
Contact: *Dr. J. P. Duran*
Gran Via de les Corts Catalanos
794 Bajos
Barcelona 13, Spain

October 30-November 2, 1982

Title: *6th Annual Symposium on Computer Applications in Medical Care*
Location: *Washington, D.C.*
Contact: *Bruce I. Blum*
SCAMC-Office of CME
2300 K Street, N.W.
Washington, D.C. 20037
Telephone (202) 676-4285

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September 1982, Volume VI, No. 9

U.S. DEPARTMENT OF HEALTH
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Serving Animal Resources,
Biotechnology Resources,
General Clinical Research Centers,
Minority Biomedical Research Support,
and Biomedical Research Support

This Issue:

- Damage from DMSO
- Pigeon Breeders' Disease
- New SLE Model
- AGI Inhibitor

by
Greg Freiherr

ESR Spectroscopy: A Tool for Probing the Cell

Measurements of the absorption of microwave energy by unpaired electrons in biomolecules are enabling researchers to chart the structure and function of cells, uncovering information crucial to understanding cell biology, to defining disease, and to developing or improving therapy.

With the technique called electron spin resonance (ESR) spectroscopy researchers can describe how the function of a cell changes during division and how a cell reacts to environmental stimuli. They can measure cell respiration and the permeability of cell membranes, document the properties of specific cell materials, and study complexes that form between cell materials and biologically active metals, including iron and copper.

Laboratories around the world employ ESR spectroscopy, but the National Biomedical Electron Spin Resonance Spectroscopy Center in Milwaukee, Wisconsin, has developed its use to a highly advanced degree. Founded in May 1976 and currently directed by Dr. James Hyde, codirector of the radiation biology and biophysics section in the

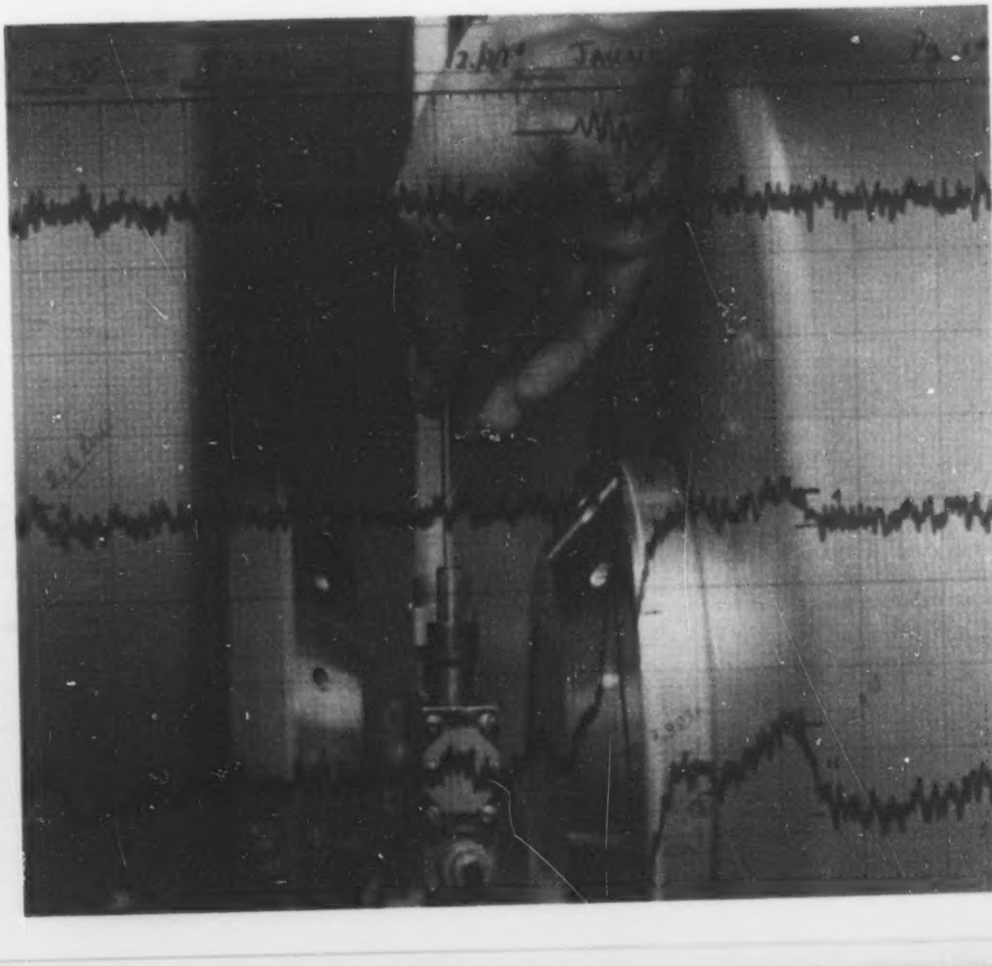

Photo courtesy of Medical College of Wisconsin

Electron spin resonance spectroscopy: ***probing the molecular interactions of life.***

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ESR *continued*

radiology and radiation oncology departments of the Medical College of Wisconsin (MCW), the center applies techniques requiring special, complex instruments. The staff develops new ESR instruments and methods, conducts research into cell biology, provides training and working space to extramural investigators, and runs analyses for those who cannot visit the center.

The science applied at the center is based on the principles that govern electromagnetism and quantum mechanics. Cell samples are placed in an intense magnetic field and bathed in microwaves. The radiation does not harm the cell. Rather, it induces transitions between the energy levels of unpaired electrons that occur, for example, in free radicals, transition elements of the periodic table, and molecular oxygen. Instruments record absorption of radiation as a function of the magnetic field. Resulting spectra are interpreted to determine the identity, structure, motion, and concentrations of molecules containing the electrons.

"The beauty of ESR spectroscopy is that it provides very specific information about the cell," Dr. Hyde says. "Other types of spectroscopy cannot provide this specificity."

Radiation such as ultraviolet (UV) or infrared light, both commonly used in spectroscopy, is absorbed by molecules throughout the cell, resulting in very poorly resolved spectra composed of myriad overlapping signals. "Specificity is gained by the use of chemical tracers or probes that are sensitive to ESR spectroscopy," Dr. Hyde says.

With ESR, molecules such as the pigment melanin, which is present in skin cells, serve as intrinsic probes. Certain molecules modified to reside in specific areas of the cell after they are introduced into the cell serve as extrinsic probes.

"You need to be clever in using the probe, whether it is extrinsic or intrinsic, and clever in chemically constructing the extrinsic probe and getting it into the right place in the cell," Dr. Hyde says. "When this is done well, the probe acts as a reporter, sending back specific information about the cell. Depending on the circumstances, we can determine cellular microviscosity and chemical structure in the vicinity of the probe, oxygen concentration, receptor binding, membrane transport, and other cellular properties."

Because cells are suspended in solution, they continue to function during examination. Much current research is directed toward studying transient cellular events initiated by light, heat, or sudden change in the fluid surrounding the cells, which is caused by rapid mixing of the cellular suspension with a different fluid.

The application of ESR spectroscopy to biomedicine is research at the most basic level, according to Dr. Hyde. The technique can be used to explore the mechanism by which materials diffuse across the cell membrane, which is both a barrier and a transportation system. The membrane keeps certain potentially harmful materials out of the cell, while permitting nonharmful materials including oxygen, hormones, and nutrients to enter. The permeability or fluidity of the membrane, therefore, influences the activity of the cell. If the membrane is damaged and prevents the cell from obtaining adequate nutrients or from excreting metabolites, the cell functions abnormally and may die.

Experimental evidence indicates that certain diseases affect membrane permeability in ways that may impair cell function. A better understanding of the cell membrane, therefore, may yield a better understanding of disease. It may also point the way to improving the action of drugs that must traverse membranes to reach their targets.

A major determinant of membrane permeability is the motion of molecules within the membrane, which is composed largely of lipids that form two adjacent layers. The lipids extend from the surface of each layer to the middle. Lipid segments near the middle undergo more rotational and wobblelike motions than those near the surfaces and act as though they are suspended in fluid, a phenomenon that gave rise to the concept of a fluidity gradient. Quantitative measurement of this gradient by ESR characterizes the increase in molecular motion from the surfaces of the membrane to the middle.

Variations in fluidity are thought to result from the presence of certain proteins, Dr. Hyde says. Random motions, classed as either rotational diffusion or lateral diffusion, are thermally driven and change when the temperature in the membrane changes. Molecular motion has a profound effect on the rates at which materials can enter or leave the cell.

To study this motion, and thereby obtain a better understanding of membrane permeability, researchers at the ESR center attach chemical tags to lipids, cholesterol, and proteins normally present in membranes. Rotational diffusion of these tags is indicated by the widths of ESR spectral lines. Broad lines show little motion. Very narrow lines show a lot of motion.

To assess lateral diffusion, spin labels, which do not combine with other molecules but freely diffuse throughout the membrane to reflect an average property of the sample, are introduced. Collisions between spin labels themselves and between spin labels and molecular oxygen give rise to observable changes in the ESR spectra that reflect this type of diffusion.

Since advanced knowledge about the cell, and particularly its membrane, promises enormous benefits to the biologist and the clinician, this area of study is strongly emphasized at the Milwaukee ESR center. Most work begins by defining basic molecular interactions in artificial membranes. The next step is to study membranes of cells cultured in vitro.

The primary experimental system used by the Milwaukee researchers has been the Chinese hamster ovary cell, which is relatively easy to culture. Changes in membrane fluidity during normal growth are documented. With this knowledge, the effects of external factors such as radiation, heat, disease, or drugs can be assessed. These factors often push the cell in an abnormal fashion to various phases of development.

Dr. Ching-San Lai, assistant

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professor of biophysics at MCW, and his colleagues have developed an ESR method to measure oxygen uptake by the cell. They have demonstrated with the ESR method that the amount of oxygen absorbed by the cell changes when the cell reproduces. Dr. Lai is now investigating the effects of temperature and various growth conditions on cell respiration.

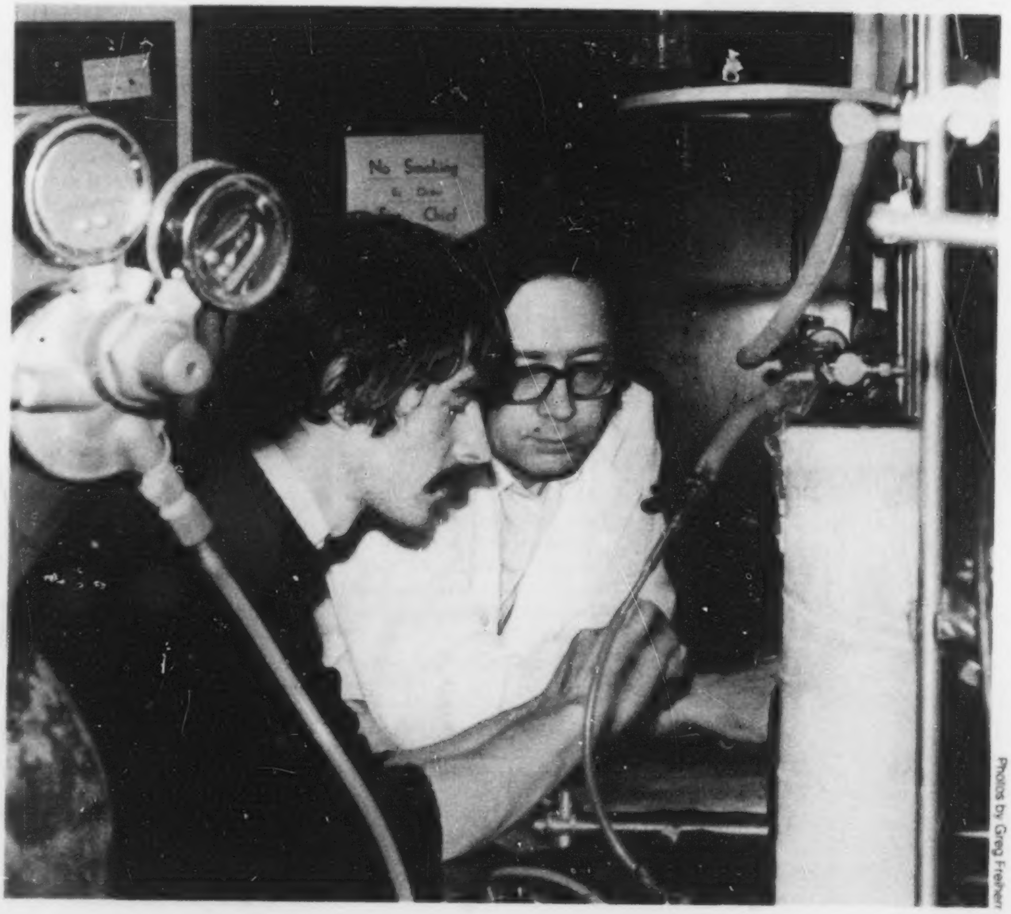
By understanding the mechanism of membrane transport, researchers may find ways to alter the uptake of oxygen in specific cells. Methods of decreasing or increasing the diffusion of oxygen could help repair diseased or damaged cells.

These methods might also be used to influence the uptake of oxygen by cancer cells, an action that some researchers believe could make cancer tumors more vulnerable to radiation. It is known, for example, that cells near the center of a tumor and far away from the blood supply are resistant to radiation. The resistance, some physicians suspect, is due to a lack of oxygen in the cells.

Acting on this assumption, some radiotherapists prefer to administer several doses of low-energy radiation intermittently over a period of hours. Dr. Larry E. Hopwood, codirector with Dr. Hyde of the radiation biology and biophysics section in the MCW radiology and radiation oncology departments, believes that oxygen tension at cells in tumor layers beneath cells already killed by low-dose radiation increases between treatments. Increasing the oxygen concentrations might raise the susceptibility of tumor cells to subsequent radiotherapy, Dr. Hopwood says. Some physicians also prescribe drugs that mimic the action of oxygen when it enters cancer cells. These drugs are believed to sensitize the cancer to radiation.

The hypothesis that oxygen increases the susceptibility of cells to radiation is supported by the favorable response of patients receiving this type of drug with a regimen of radiotherapy, says Dr. Hopwood, who is an adviser

Dr. James Hyde, director of the National Biomedical ESR Spectroscopy Center: *"The beauty of ESR spectroscopy is that it provides very specific information about the cell. Other types of spectroscopy cannot provide this specificity."*



Researchers at the National Biomedical Electron Spin Resonance Spectroscopy Center in Milwaukee: *probing the cell with microwaves.*

Photos by Greg Frehen

ESR *continued*

on cell biology to researchers at the ESR center. The only direct support for the hypothesis, however, comes from animal studies, since humans could not be exposed to the sampling procedures necessary to document changes in the use of oxygen by tumor cells.

Another factor may influence the susceptibility of cancer cells to radiation and the action of drugs. According to Dr. William Antholine, a biophysicist at MCW, the killing power of some antitumor drugs increases significantly when certain metals are bound to the drug molecule. Animal research indicates that when such binding occurs, the drug becomes much more effective.

ESR allows Dr. Antholine to confirm that the metals are bound to the drug in cells and to identify the number of nitrogen atoms from the drug that are bound to the metal. ESR also provides a detailed account of the cellular metabolism of the drug.

"The data for monothiosemicarbazone agents are spectacular," Dr. Antholine says. "Without the copper complex, antitumor agents show only a moderate killing effect. When the metal is added, cancer cell survival decreases dramatically in proportion to the metal concentration. And if drug administration is followed by radiotherapy, the effect is heightened even more."

Dr. Antholine and associate professors of chemistry Drs. Judith Knight and David Petering at the University of Wisconsin-Milwaukee initially demonstrated the potential of these preformed metal complexes with experiments on Ehrlich ascites tumor cells. More recently Drs. Antholine and Hopwood learned that the mean cell lethal dose of the thiosemicarbazone antitumor drug bound to copper and administered to Chinese hamster ovary cells is 25 times lower than without the metal.

The research has been expanded to include metal derivatives of thiosemicarbazone and the antitumor agent bleomycin. Both combine with copper or iron. Dr. Antholine's primary interest now is to determine whether metal complexes in these agents sensitize cancer cells in animals with induced tumors to radiation. In this project the effects of radiation and drugs with and without metal complexes are individually recorded and compared to the effects of combining these drugs with radiotherapy.

The physical chemistry and experimental methods used in these studies can also be employed in the study of metal-binding properties in a wide variety of biomolecules, including hemoglobin. Research with ESR on transition metals such as iron or copper is one of the most active areas of its application, Dr. Hyde says.

Because even common forms of radiation such as UV light affect living tissue, Dr. Christopher Felix, a chemist and manager of the ESR center, and Dr. Roger Sealy, associate professor of radiology and biochemistry at MCW, are examining how melanin protects cells from the harmful effects of UV radiation. Scientists know that the more melanin there is in a person's skin, the less likely that person is to be sunburned. A person with a high level of melanin is also less likely to develop skin cancer.

The research may lead to the improvement of methods for avoiding the painful effects of sunburn, lessening the chance of skin cancer by exposure to sunlight, and developing alternate therapy for some skin diseases that are now treated with certain wavelengths of UV light.

Dr. Felix and his colleagues began their research by subjecting isolated melanin pigments to UV light and other radiation and using ESR to record the response of melanin. They know that oxygen is necessary for pigment darkening, which occurs immediately after exposure to radiation. Their experiments showed that melanin takes up oxygen during irradiation and that the oxygen is converted into hydrogen peroxide. Similar oxygen consumption, induced by exposure to light, occurs in cultured mammalian cells containing ingested melanin.

The researchers believe that this oxidation may be protective, drawing oxygen from other, nonpigmented cells in which radiation otherwise could convert oxygen molecules to singlet oxygen or superoxide radicals. They explain that both forms of oxygen are highly toxic and can cause cell death or mutations

Dr. Ching-San Lai, assistant professor of biophysics at the Medical College of Wisconsin (MCW): ***measuring oxygen uptake by the cell.***

Dr. Larry E. Hopwood, codirector of the radiation biology and biophysics section at MCW: ***will increasing oxygen uptake by cancer cells make them more susceptible to radiation?***

associated with the development of skin cancer. Cell enzymes can detoxify superoxide, but massive production of superoxide overwhelms these enzymes and cell damage results.

The Milwaukee group is now correlating specific chemical reactions with the wavelength and the intensity of the irradiating light. Oxygen uptake along with the concentrations and lifetimes of reactive free radicals formed during chemical reactions of the pigment are also being studied with ESR. Resulting data are being compared with the quantity of melanin present in the cell and the length of time cells survive under continued exposure to light.

In a separate project, Dr Sealy is studying the oxidative effects of certain enzymes and of light on catecholamines, compounds such as epinephrine, norepinephrine, and dopamine, which function as hormones or neurotransmitters. Free radicals formed during the oxidation of catecholamines lead to the production of *o*-quinones, which are highly toxic.

ESR is used to identify the radicals and to determine their rates and mechanism of formation and decay. Very low levels of radical formation can be detected by stabilizing the radicals with metal ion complexes. The information may lead to ways of designing less toxic drugs derived from catecholamines.

Ongoing studies at the Milwaukee ESR center are examples of the potential that electron spin resonance spectroscopy holds for medical and biological researchers. Although the technique has been used by physicists and chemists for many years, only within the past decade has it been extensively applied in the biomedical arena. "Application of ESR to cell biology and to investigations of disease and therapy at a cellular level are just beginning," Dr. Hyde says.

Additional reading:

1. Lai, C. S., Hopwood, L. E., and Swartz, H. M., ESR studies on membrane fluidity of Chinese hamster ovary cells grown on microcarriers and in suspension. *Experimental Cell Research* 130: 437-442, 1980.
2. Lai, C. S., Hopwood, L. E., and Swartz, H. M., Electron spin resonance studies of changes in membrane fluidity of Chinese hamster ovary cells during the cell cycle. *Biochimica et Biophysica Acta* 602:117-126, 1980.
3. Popp, C. A., and Hyde, J. S., ELDOR and saturation recovery studies of nitroxide electron and nuclear spin-lattice relaxation times and Heisenberg exchange rates: lateral diffusion in dimyristoylphosphatidylcholine. *Proceedings of the National Academy of Sciences U.S.A.* 79: 2559-2563, 1982.
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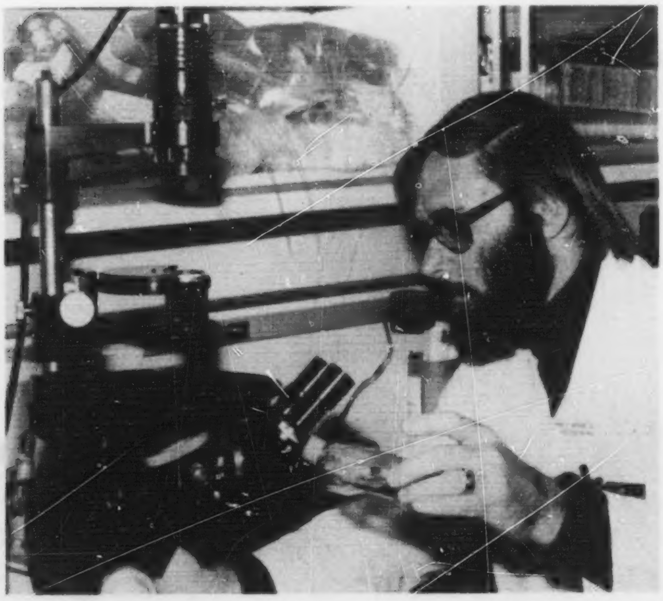


Funding for research into membrane permeability, antitumor drugs, melanin, and catecholamines is provided by the American Cancer Society, the National Cancer Institute, the National Institute of General Medical Sciences (NIGMS), the National Science Foundation, and the Biotechnology Resources Program (BRP) of the NIH Division of Research Resources. The National Biomedical ESR Center in Milwaukee is supported by BRP, NIGMS, and the Medical College of Wisconsin.

Research Focus

DMSO Induces Chromosomal Damage in Cell Cultures

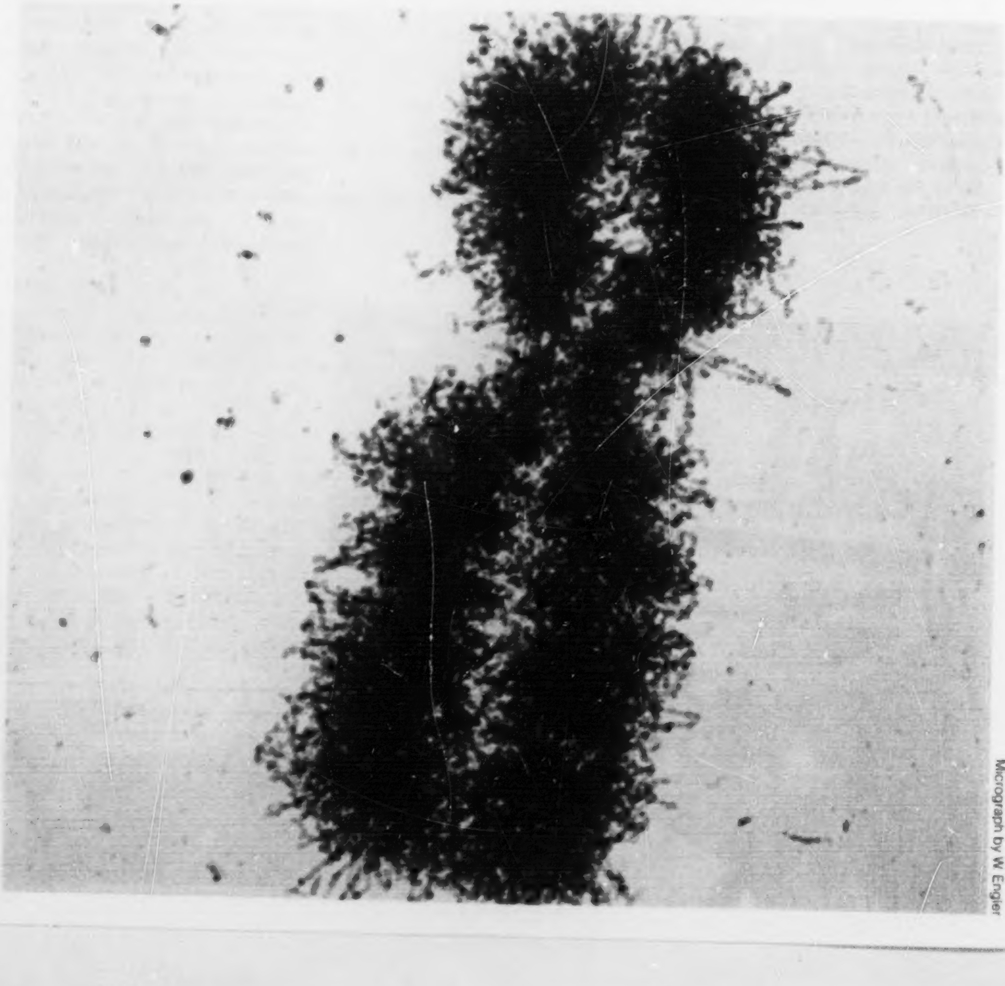
Dimethyl sulfoxide (DMSO), a chemical sometimes used by arthritis patients and athletes as a local remedy for aches and pains, caused significant chromosomal damage in Chinese hamster ovary (CHO) cell cultures in a study conducted by researchers at Atlanta University in Georgia.

Although the drug is not approved by the U.S. Food and Drug Administration for this type of treatment, some patients use it as a home remedy without physician's approval. DMSO is a byproduct in the manufacture of paper and is widely used as an industrial and laboratory solvent. When applied to the skin, it is quickly absorbed and circulates generally in the body tissues and fluids.

DMSO concentrations of 1 to 4 percent increased chromosomal aberrations up to 27 percent, compared to 5 percent increased chromosomal aberrations in control cultures not exposed to DMSO. Dr. Rosalyn M. Patterson and Mr. Earl Smalls of the biology department used a highly purified spectroscopic grade of DMSO in the study.

The extent of chromosomal aberration was dose-dependent. At levels of 2, 3, and 4 percent DMSO, the ratios of aberrations after 24 hours were 14, 25, and 27 percent respectively. Dr. Patterson points out, however, that when the cells were exposed to DMSO for 48 hours and then maintained in culture for 8 days without the chemical, the percentage of chromosomal aberrations was not significantly greater than in the untreated control cultures. "These results may be due to DNA repair mechanisms or to the death of severely damaged cells," she says.

Centromere separations, chromatid gaps and breaks, and triradials were the most frequent aberrations detected after the 4 percent dose.Chromatids are the two spiral filaments, joined at a point called the centromere, that make up a chromosome. Chromatids separate at cell division, and each becomes a chromosome in one of the daughter cells. Triradials are three chromatids joined together and involve translocations from at least two chromosomes, Dr. Patterson explains. Other aberrations frequently encountered in the study were chromatid fusions, dicentrics, and polyploids.



Human chromosome composed of sister chromatids, spiral filaments joined at the centromere: *targets of damage from DMSO.*

Micrograph by W. Engler

DMSO is oxidized to dimethyl sulfone ($DMSO_2$) and reduced to dimethyl sulfide in the human body. Metabolism apparently is not complete, however, since both DMSO and $DMSO_2$ are excreted in the urine and feces. Dimethyl sulfide is eliminated through the breath and skin and is responsible for the characteristic garlic-like odor from patients on DMSO medication.

Sister chromatid exchanges (SCE's) were also found in CHO cell cultures exposed to low levels of DMSO. SCE's are exchanges of DNA units between the two chromatids, or sister chromatids, on the same chromosome. Differences between exchange frequencies in treated and control cultures varied with dosage to a statistically significant degree. Mutagenic agents often significantly increase the frequency of exchanges between sister chromatids, Dr. Patterson says.

The powerful solvent and penetrating action of DMSO may pose a greater threat to the patient than the chemical itself, according to Dr. Patterson. She points out that DMSO applied locally could carry environmental chemicals, deposited on the skin from various sources, into the body. This could be especially dangerous in factory workers exposed to toxic chemicals.

Research has shown that DMSO also causes a variety of adverse effects in experimental animals, Dr. Patterson says. For example, a significant increase in chromosomal aberrations in the bone marrow cells of rats was found in animals injected intraperitoneally with varying doses of DMSO.

Congenital defects have been produced in chick embryos, rats, mice, and hamsters by high doses of the compound. "Although these malformations occurred at low frequencies, the potential for induction of adverse embryological effects by DMSO has been established," Dr. Patterson says.

Eye defects have also been observed in DMSO-treated animals. Changes in the refractive index as well as opacity of the lens were found in monkeys, dogs, and rabbits given high doses of the compound over an extended period of time. Allergic-like reactions also have been observed occasionally in individuals using DMSO topically.

Dr. Patterson believes that more extensive, long-term investigations should be conducted with cultured cells and laboratory animals to obtain more information. "Particular attention should be given to the effects of DMSO on chromosomes of the blood-forming organs and on cells of the ovaries and testes," she says.

"Since DMSO can be absorbed directly through the skin and is often applied locally on joints, it is likely that the compound is transported by the blood, without significant metabolic alteration or detoxification of the DMSO, directly to those organs which may be most seriously affected."

Additional reading:

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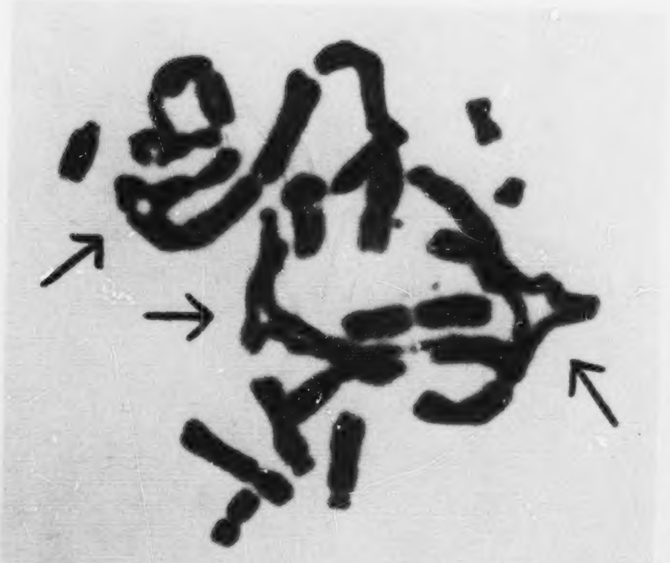
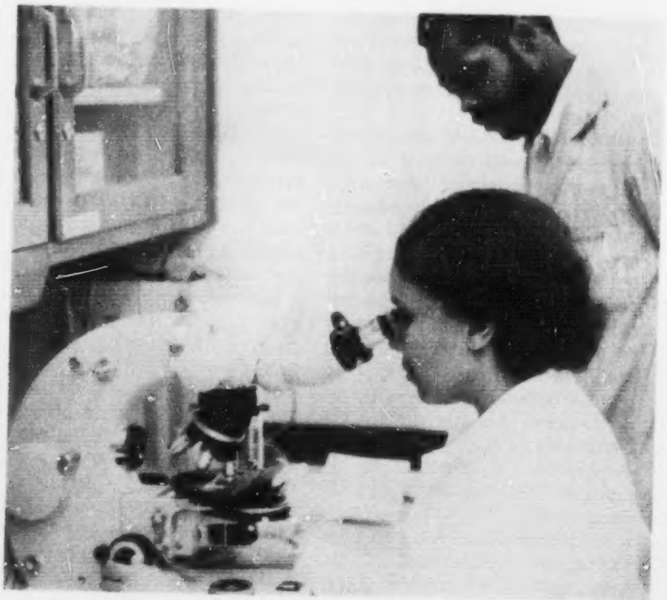
Research into dimethyl sulfoxide-induced chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells was supported by the Minority Biomedical Research Support Program of the NIH Division of Research Resources.

Chromosomal aberrations in cells treated with DMSO, a folk remedy for joint pain: *genetic danger for the self-prescriber.*



Dr. Rosalyn Patterson and Mr. Earl Smalls: *studying the effect of DMSO on chromosomes in Chinese hamster ovary cells.*



Pigeon Breeders' Disease: Model For Some Allergic Lung Disorders

The triggers of allergic lung disease are in the home and in white collar businesses, in grain silos and in freight cars, even in pigeon cotes. Regardless of the source, the effects are always the same—impaired breathing, coughing, fever, and chills—the symptoms of a broad class of disorders called hypersensitivity pneumonitis.

Some people exposed to these allergens develop symptoms and others do not. The reason, according to researchers at the Medical College of Wisconsin (MCW) in Milwaukee, is that some people maintain a natural defense, cells that specifically suppress the effects of allergens. When these cells, called suppressor T-lymphocytes, fail to suppress the immune response, antibodies and sensitized cells attack and damage lung tissue, leading to the symptoms of hypersensitivity pneumonitis, says Dr. Jordan Fink, chief of the allergy and immunology section in the MCW department of medicine.

In studying patients with pigeon breeders' disease, a lung allergy caused by inhaled pigeon matter, Dr. Fink and his colleagues at MCW discovered that a population of suppressor cells is active in the blood of healthy pigeon breeders but not in the blood of patients with symptoms of the allergy. Although pigeon breeders' disease affects only a small number of people, it may be a model for all forms of hypersensitivity pneumonitis. "The symptoms are identical for all types of this disorder," says Dr. Fink.

If his conclusion is correct, the Wisconsin research has broad applicability. According to Dr. Fink, a variety of bacteria, fungi, and protozoa can cause hypersensitivity pneumonitis. These microorganisms can invade virtually every segment of society. They can live in the heating and cooling systems of homes or businesses, contaminating the air breathed by residents or employees. They may be present in foodstuffs such as grain and malt, exposing farmers and handlers on loading docks and in breweries to airborne allergens.

Scientists have long suspected that a defective immune system, triggered by the presence of allergens, misdirects antibodies against host tissue, producing the symptoms of hypersensitivity pneumonitis. Studies by the Milwaukee investigators showed that this suspicion is false. No significant difference in the level of circulating antibodies specific to pigeon antigens was found in pigeon breeders with or without symptoms of the disease.

Pulmonary lavage revealed, however, that the lungs of symptomatic pigeon breeders contain more lymphocytes than the lungs of asymptomatic subjects—an apparent contradiction of the hypothesis that suppressor T-cells determine susceptibility to pigeon breeder's disease. Dr. Fink speculates, however, that this population is largely composed of lymphocytes drawn to the lungs by the attack of antibodies on lung tissue and by the resulting inflammation. "Preliminary evidence suggests," he says, "that the activity of suppressor T-cells in the lungs of symptomatic pigeon breeders is not adequate to control the immune response to pigeon antigens." He cautions, however, that lavage material from only a few patients has been closely examined and that definitive conclusions cannot yet be drawn.

At present the hypothesis that the activity of suppressor cells is the major determinant of hypersensitivity pneumonitis in pigeon breeders is primarily supported by tests indicating greater suppressor cell activity in the blood of asymptomatic subjects than in the blood of patients with clinical signs of the disease. Dr. Fink believes this heightened activity in asymptomatic subjects occurs throughout the body.

If his hypothesis proves to be correct, the research may lead to an effective treatment or cure for hypersensitivity pneumonitis. Currently the preferred therapy is to avoid an allergen once it is identified. But that is not always possible.

"In pigeon breeding, which is a hobby, changing the environment is relatively easy," says Dr. Fink. "But for the farmer, who is regularly exposed to allergens, avoidance is seldom possible. It's his career. Farmers can reduce contact with suspect microorganisms by special equipment and handling procedures, which have recently diminished the incidence of farmer's lung in Wisconsin, but not all farmers have the money to take these measures."

Economic factors must be considered in other areas as well, he says. If just a small number of workers are affected by contaminated air in a business office, the heating and cooling system has to be replaced, the business has to move or pay workmen's compensation, or the workers have to find new jobs.

The only effective treatment currently available for hyper-

Dr. Jordan Fink at the Medical College of Wisconsin: *studying the cells that supply the immune response to allergens.*

Mr. Carl C. Gill, pigeon breeder and racer in Milwaukee, Wisconsin: *recruiting fellow hobbyists to participate in allergy tests at the Milwaukee GCRC.*

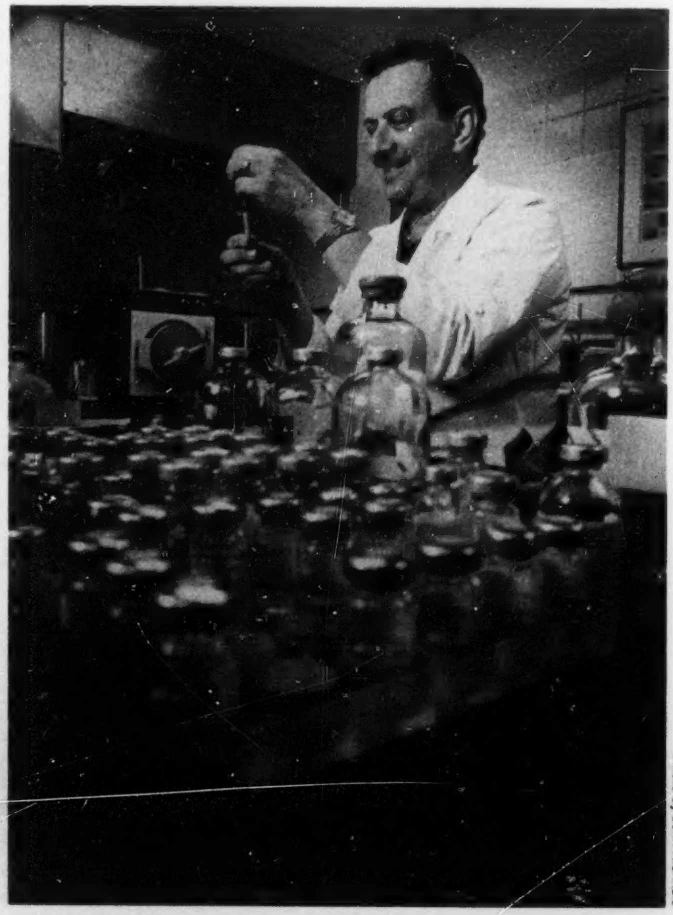


Photo by John Nienhuis

sensitivity pneumonitis is the use of corticosteroids, which can cause hypertension, decalcification of bone, and numerous other adverse effects when used for a long time. To make management even more difficult, neither avoidance nor treatment with corticosteroids is always enough. Symptoms may persist in some patients, leading to chronic lung disease, which reduces the patient to a "pulmonary cripple," says Dr. Fink.

With pigeon breeders' disease as a model, the Milwaukee researchers hope to understand the defect that inactivates suppressor cells and makes people susceptible to allergens. Although the research team is studying various forms of hypersensitivity pneumonitis, pigeon breeders' disease is particularly attractive to them because pigeon antigens are relatively few and easy to manipulate in the laboratory, compared to the large numbers of antigens released by microorganisms associated with other forms of lung hypersensitivity. Patients with pigeon breeders' disease show clear-cut signs of the disease, thereby simplifying the diagnosis. And pigeon breeders want to participate in the research, providing financial support through their national organization and traveling across the nation to Milwaukee for tests conducted at the MCW General Clinical Research Center (GCRC).

The GCRC is a base from which the researchers can work. It provides a support staff and a controlled environment in which patients can recuperate from pulmonary challenges necessary to confirm the diagnosis and examine the mechanism of the disease.

In continuing their study of pigeon breeders' disease, the first priority of Dr. Fink and his colleagues is to design a screening method for identifying susceptible individuals. Their next priority is to design a therapy, one that perhaps will reactivate the protective mechanism that is dormant in patients with the disease.

Additional reading:

1. Moore, V. L., Pedersen, G. M., Hauser, W. C., and Fink, J. N. A study of lung lavage materials in patients with hypersensitivity pneumonitis: in vitro response to mitogen and antigen in pigeon breeders' disease. *Journal of Allergy and Clinical Immunology* 65:365-370, 1980.
2. Scribner, G. H., Barboriak, J. J., Fink, J. N., Prevalence of precipitins in groups at risk of developing hypersensitivity pneumonitis. *Clinical Allergy* 10:91-95, 1980.
3. Fink, J. N., Immunologic lung disease. *Hospital Practice* 16: 53-63, 1981.

The study of pigeon breeders' disease at the Medical College of Wisconsin GCRC is funded primarily by the National Heart, Lung, and Blood Institute, the National Institute of Allergy and Infectious Diseases, and the Veterans Administration. The GCRC is supported by the General Clinical Research Centers Program of the NIH Division of Research Resources.

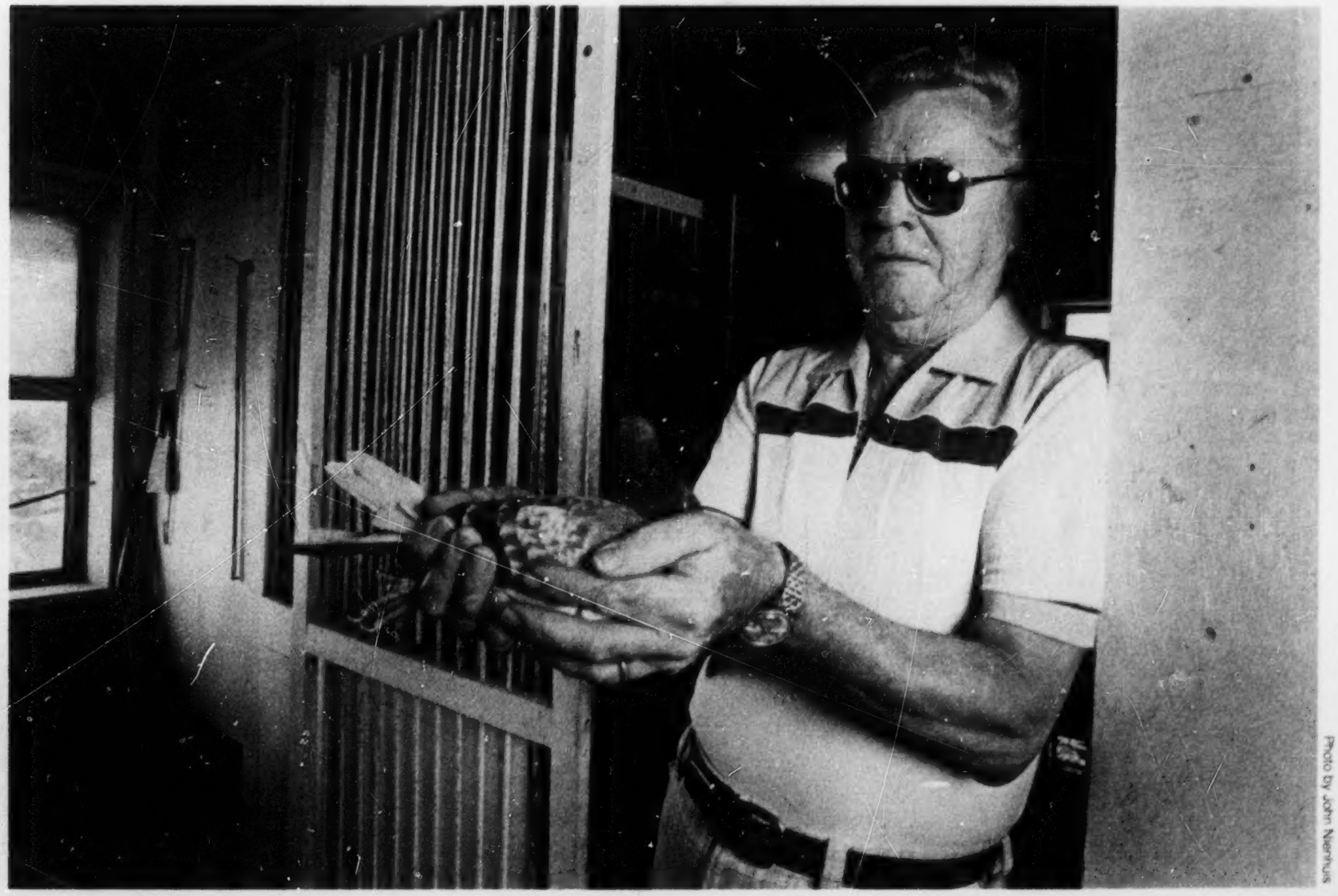


Photo by John Nienhuis

Weight Loss With Fat Substitute

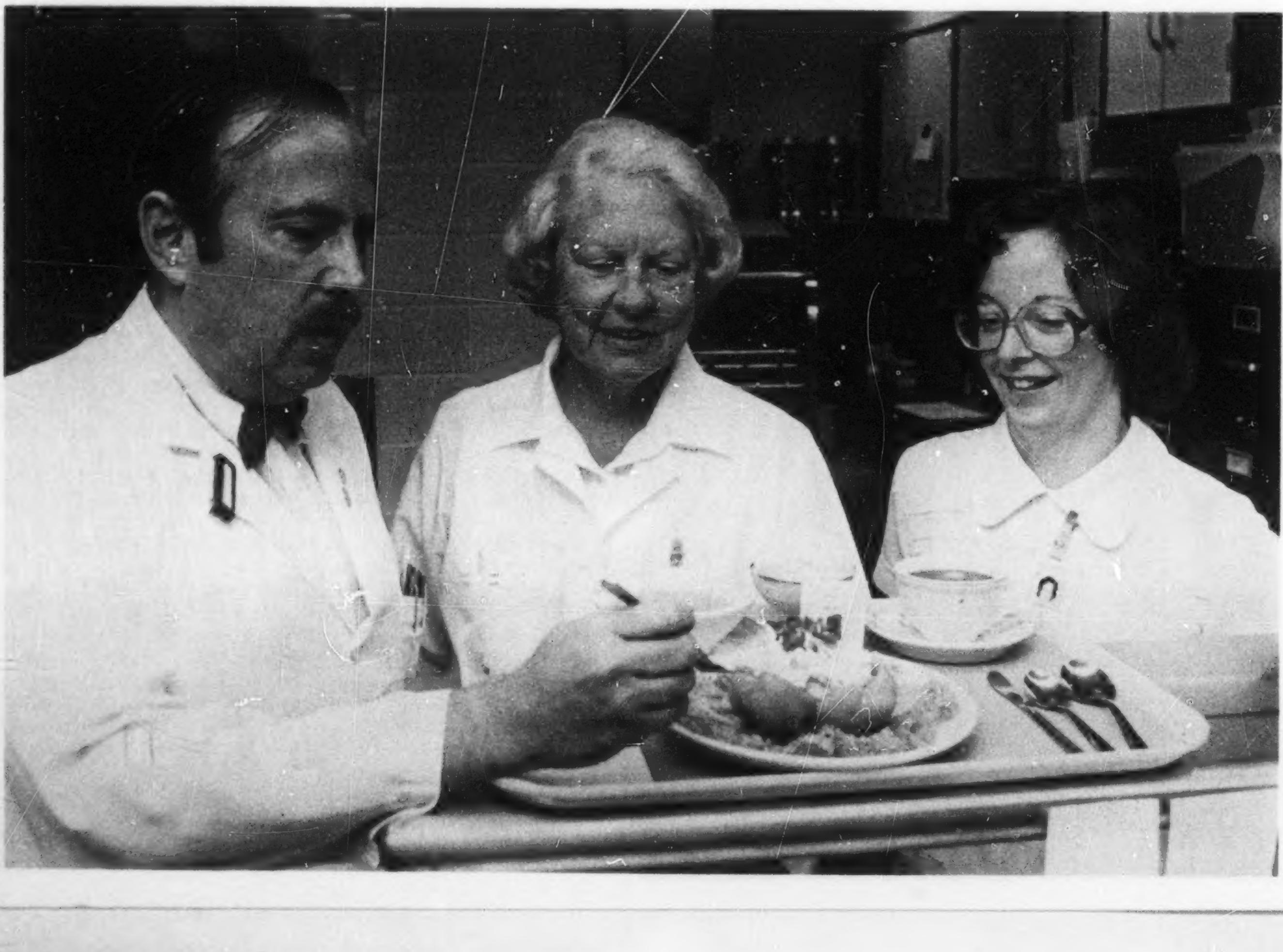
A fat substitute called sucrose polyester (SPE) has been tested as an aid to losing excess weight.

Because the experimental drug cannot be absorbed by the digestive tract, and because it tastes, looks, and smells like common dietary fats, investigators at the University of Cincinnati were able to induce weight loss without reducing the quantity or affecting the flavor or appearance of food eaten by their patients. According to Dr. Charles J. Glueck, chief researcher, the subjects in the study did not realize that they were eating fewer calories. The result from eating a diet containing the fat substitute was an average weight loss of nearly 0.4 pounds per day over a period of 20 days.

"Sucrose polyester is not a panacea for all obesity," Dr. Glueck cautions, "but it is a new, powerful tool to help bring about sustained weight loss in obese individuals, which in my opinion is the most difficult metabolic intervention to make and sustain in clinical medicine." Having reached the desired weight, patients might continue on a maintenance program with SPE to prevent the weight gain that usually follows completion of a diet, he explains.

The only adverse effects noticed during the use of sucrose polyester were reductions in the serum levels of vitamins A and E. The changes were barely perceptible and may not have truly indicated a drop in serum vitamin levels, according to Dr. Glueck. Measurements might have reflected changes in levels of nonvitamin compounds that respond to the methodology employed by the Cincinnati research group. Specific assays for vitamin A, which were done on selected patients, revealed no change in the serum level of the vitamin.

Because vitamins A and E are fat soluble, however, serum levels of the vitamins could be affected by changing the intake of dietary fat. The chance that SPE causes such changes is a concern of Dr. Glueck and his colleagues. In past and current studies the researchers have closely monitored these vitamins. Dr. Glueck emphasizes that general or specific tests on patients



Dr. Charles Glueck samples a menu with foods made from the experimental cooking substance sucrose polyester, which holds promise as an effective weight-loss agent. With Dr. Glueck are Elizabeth Hogg (center) and Charalee Allen, nutritionists at the University of Cincinnati General Clinical Research Center, where sucrose polyester recently underwent clinical testing. Dr. Glueck is director of the center, one of 72 such units throughout the United States.

being treated with SPE for up to 40 days have not revealed any biologically significant changes to date.

The fat substitute offers advantages other than weight loss. Previous studies conducted by Dr. Glueck and his colleagues at the University of Cincinnati demonstrated that sucrose polyester may retard atherosclerosis, or hardening of the arteries. The compound significantly reduces total plasma cholesterol and low-density lipoprotein cholesterol and the intestinal absorption of cholesterol. (See *Research Resources Reporter*, June 1978 and January/February 1980.)

The most recent study of SPE as an aid for weight loss was conducted with 10 chronically obese subjects who were hospitalized in the Cincinnati General Clinical Research Center, which is directed by Dr. Glueck. "The study required a minutely controlled metabolic kitchen run by research-oriented nutritionists," he says. "It also required a nursing staff skilled in research techniques, as well as in patient care, and a laboratory with a high degree of quality control."

Patients were examined initially for 7 to 14 days to determine baseline intake of calories. In the experiment that followed, patients served as their own controls, first eating a normal diet before switching to one with SPE substituted for dietary fat, or vice versa. Each diet was followed by each subject for 20 days. Until the end of the study, neither the researchers nor the patients knew when the fat substitute was being eaten.

An average of 60 grams of SPE per day replaced conventional fat in the altered diet—a reduction of 540 calories per day. Both the SPE diet and the diet containing only normal foods required 60 percent of the baseline calorie intake at mealtime. Patients could ingest an additional 60 percent of their baseline intake by eating snacks, which were available at a specific time each day. If they chose to eat the snacks, they could possibly gain weight or at least maintain their weight.

Dr. Glueck reports that total calorie intake, which included meals and snacks, fell by an average of 23 percent with the sucrose polyester diet. Subjects did not report increased hunger during the SPE diet period—a subjective measure supported by the fact that they did not increase their eating of snacks.

"The subjects clearly were better able to reduce their total intake of calories during the SPE period, while at the same time demonstrating appetite satisfaction—a central key to continuing a diet," says Dr. Glueck. In addition, data from the period during which patients were fed SPE demonstrated a 10 percent reduction in total plasma cholesterol and a 14 percent reduction in low-density lipoprotein cholesterol, the cholesterol fraction mainly associated with the development of atherosclerosis. There was also a 10 percent reduction in the triglyceride level, another factor associated with atherosclerosis.

During the study, sucrose polyester was administered in three forms: a semisolid mixture containing 80 percent SPE oil and 20 percent hydrogenated palm oil, which was used in salad dressings and other recipes; a sandwich spread containing 54 percent sucrose polyester oil, which has a flavor and consistency similar to corn oil margarine; and a mixture of SPE and nonfat milk that resembled a milkshake.

By substituting sucrose polyester for cooking fats, a 3-ounce serving of roast beef and gravy is reduced from 362 calories to 198 calories, while a 150-gram bowl of mushroom soup drops from 124 calories to 79. Toast and margarine have 97 calories, but when sucrose polyester replaces the margarine, the combination has only 61 calories. A 170-gram vanilla milkshake contains 235 calories, but with sucrose polyester substituted for milk fat it has only 95.

Sucrose polyester, an investigational new drug, has not yet been approved by the Food and Drug Administration (FDA) for general use. Dr. Glueck declines to speculate on when the drug will be approved. "Investigational new drugs must overcome a series of efficacy and safety hurdles mandated by the FDA," he says. "These hurdles involve human studies and it is increasingly clear that we could not fully develop new drugs unless we had GCRC's. SPE, for example, could not have been developed to this point without the support of the Cincinnati GCRC and the analytical support of our CLINFO center."

CLINFO is an interactive computer system that allows researchers to record and analyze their own data. The system, available at about 11 GCRC's, is designed to be easily understood and used by clinical investigators, even if they have no prior experience with computers. (See *Research Resources Reporter*, December 1981.)

CLINFO and the Cincinnati GCRC are now being used to conduct a 7-month, double-blind study of SPE, which will involve about 100 outpatients.

Additional reading:

Glueck, C. J., Hastings, M. M., Allen, C., Hogg, E., Baehler, L., Gartside, P. S., Phillips, D., Jones, M., Hollenbach, J., Braum, B., and Anastasia, J. V., Sucrose polyester and convert calorie dilution. *American Journal of Clinical Nutrition* 35:1352-1359, 1982.

Support for the study of sucrose polyester as an aid for losing weight and reducing serum cholesterol was provided by the General Clinical Research Centers (GCRC) Program of the NIH Division of Research Resources and by the Proctor and Gamble Company. CLINFO is a service of the GCRC Program.

Research Resources Reporter
September 1982

Information Exchange

Research Highlights

A new model of systemic lupus erythematosus (SLE), a disease that affects mainly young women, has been developed by feeding alfalfa seeds and sprouts to monkeys.

The amino acid L-canavanine, which is found in alfalfa seeds and sprouts, appears to be responsible for the disease in the monkeys studied, according to researchers at the Oregon Regional Primate Research Center in Beaverton and immunologists at the Oregon Health Sciences University.

"Models of SLE have been developed in dogs and mice, but this is the first such model developed in monkeys," says Dr. M. Rene Malinow, director of the cardiovascular diseases division at the primate center. "It is also the first SLE model developed involving a food."

SLE is an autoimmune disorder that affects connective tissue. Victims may develop anemia, arthritis, fever, skin eruptions including a characteristic "butterfly rash" on the face, hair loss, and renal, cardiovascular, and central nervous system disorders. At least 500,000 Americans are now afflicted with SLE, which is involved in about 5,000 deaths annually, according to the Lupus Foundation of America, Inc., which estimates that each year about 50,000 more people develop SLE, a disease of unknown cause.

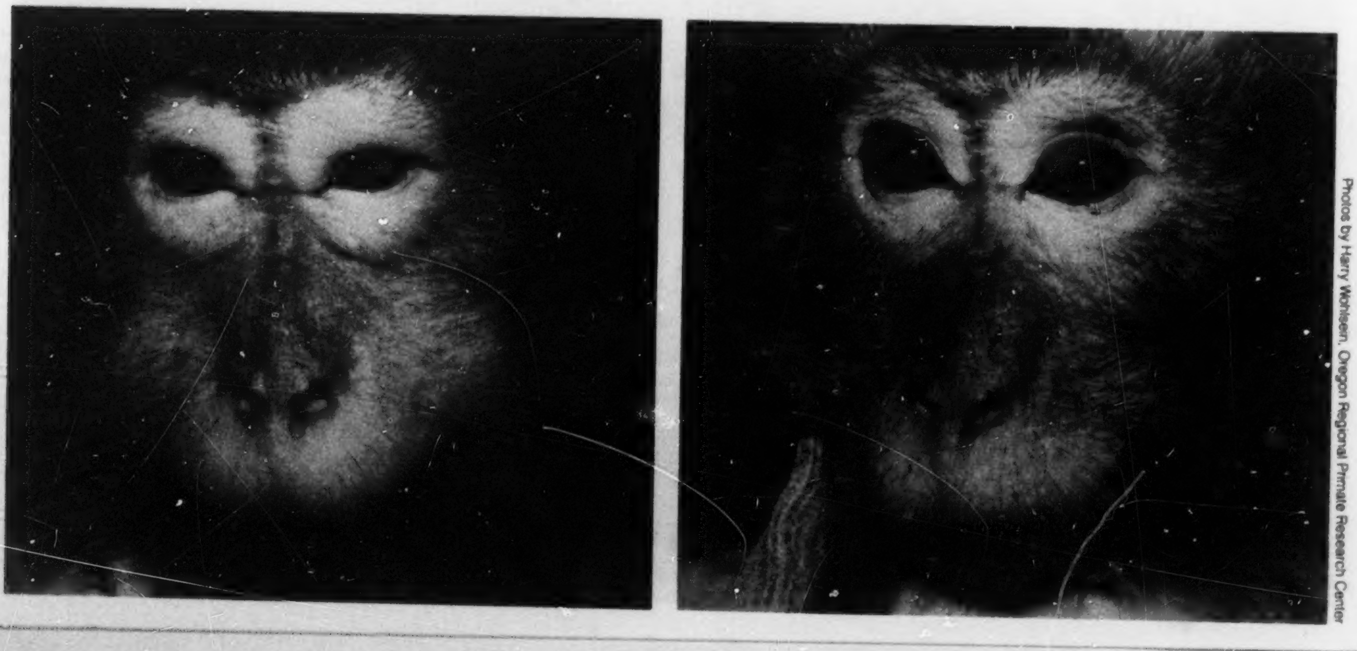
A better understanding of the autoimmune mechanism present in the alfalfa-fed monkeys may provide a clue to the workings of the disease in humans, Dr. Malinow says. Because only some monkeys develop the disease, a possible genetic susceptibility to SLE can be investigated. Another advantage of the model is that "unproven therapies difficult to test on humans can be tested on monkeys," Dr. Malinow says. Immunosuppressive drugs with unproved effectiveness against SLE can be tested in a double-blind experiment, says Dr. Emil J. Bardana, Jr., who collaborates with Dr. Malinow. Since mainly women contract the disease, the effect of male hormones on SLE can also be studied, he adds.

The Oregon researchers were originally studying alfalfa as a means of reducing cholesterol and preventing atherosclerosis. To test that hypothesis, Dr. Malinow ate between 80 and 160 grams of alfalfa seeds daily for most of a 5-month period. To his surprise, he developed hemolytic anemia, a symptom of SLE.

"I felt no pain, but what I had seemed very much like SLE," Dr. Malinow says. Subclinical symptoms included mild anemia, leukopenia, and moderate splenomegaly. Erythrocyte binding of IgG and antinuclear antibodies was detected. Double-stranded DNA antibodies were slightly increased. Complement 3 and complement 4 levels were normal, although total hemolytic complement was reduced. It took almost a year for Dr. Malinow's symptoms to subside.

To test the association between alfalfa and SLE, an experiment involving 10 adult *Macaca fascicularis* females was designed. Animals were randomly and equally assigned to two groups, one on a semipurified diet with 45 percent ground alfalfa seeds, the other without the seeds. After 5 months, three animals on the seed diet developed a syndrome similar to SLE. One of the animals died with septic complications. In a similar experiment, SLE symptoms also developed in 2 of 12 monkeys fed alfalfa sprouts.

Of the five monkeys that developed an SLE-like syndrome in the two separate experiments, three returned to normal after being placed on a conventional diet. They were then fed a semipurified diet containing a 1 percent concentration of L-canavanine sulfate, a compound incorporating the amino acid suspected of causing the syndrome. The altered diet induced anemia and other disorders characteristic of SLE. One



Facial swelling (left) after 3-month diet of alfalfa seeds was alleviated (right) by steroid treatment and return to balanced diet: *a primate model for the development of systemic lupus erythematosus.*

Photos by Harry Wohlsein, Oregon Regional Primate Research Center monkey that had developed profound anemia died after 10 weeks of L-canavanine ingestion, during which the L-canavanine sulfate dosage had been doubled for the final 3 weeks.

"Since alfalfa seeds and sprouts have induced SLE in monkeys, prudent patients with the disease should not eat these foods," Dr. Malinow says. He stresses, however, that people normally would not eat the quantity of alfalfa seeds or sprouts consumed by the monkeys that developed symptoms similar to SLE.

Although a direct toxic effect of L-canavanine or other alfalfa components cannot be ruled out, the investigators believe it is more likely that the SLE-like syndrome occurs when L-canavanine, a nonprotein amino acid, replaces the vital human amino acid arginine.

Arginyl-transfer RNA synthetase reacts with L-canavanine, incorporating the arginine look-alike into proteins. The immune system may react to abnormal canavanyl proteins by giving rise to autoantibodies seen in the SLE-like syndrome, Dr. Malinow explains. The autoantibodies and their antigens sometimes form autoimmune complexes in the kidney, causing nephritis. Canavanyl proteins may also impair messenger RNA synthesis. Once RNA metabolism is curtailed, translation of proteins necessary to replicate DNA and the production of other proteins is inhibited severely.

Researchers believe that L-canavanine may replace arginine in histones, simple proteins that often combine with nucleic acids. They are associated with DNA in chromatin, the chromosomal material of the cell nucleus. L-canavanine replacement of arginine would lower the basic pH of the histone molecule, thus possibly disturbing the histone interaction with nucleic acids and the function of chromatin. Experiments have also shown that L-canavanine inhibits histone production in cells.

The hydrolytic cleavage of L-canavanine by arginase, which yields L-canaline and urea, may be another explanation of the amino acid's role in developing the SLE-like syndrome. Experimentally, the antimetabolic properties of L-canaline and its chemical derivatives have inhibited vegetable and animal growth.

Nonprotein amino acids such as L-canavanine occur in higher plants and are similar chemically to components of mammalian proteins. According to Dr. Malinow and his associates, a question for future research is whether these amino acids when ingested induce or reactivate SLE in susceptible humans.

Additional reading:

1. Bardana, E. J., Jr., Malinow, M. R., Houghton, D. C., McNulty, W. P., Wuepper, K. D., Parker, F., and Pirofsky, B., Diet-induced SLE. In press.
2. Malinow, M. R., Bardana, E. J., Jr., Pirofsky, B., Craig, S., and McLaughlin, P., Systemic lupus erythematosus-like syndrome in monkeys fed alfalfa sprouts: role of a nonprotein amino acid. *Science* 216:415-417, 1982.
3. Malinow, M. R., Bardana, E. J., Jr., Goodnight, S. H., Jr., Pancytopenia during ingestion of alfalfa seeds. *The Lancet* I:615, 1981.
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6. Rosenthal, G. A., The biological effects and mode of action of L-canavanine, a structural analogue of L-arginine. *Quarterly Review of Biology* 52: 155-178, 1977.

Funding for the research into SLE is provided by the American Arthritis Foundation, the Collins Foundation, the Gerlinger Research Fund, the Medical Research Foundation of Oregon, the Oregon affiliate of the American Heart Association, the National Heart, Lung, and Blood Institute, and the Animal Resources Program (ARP) of the NIH Division of Research Resources. The Oregon Regional Primate Research Center is supported by ARP.

Two researchers have discovered that protamine derived from salmon sperm has the properties of an angiogenesis inhibitor. The finding might be a step toward the development of a new cancer therapy, according to the researchers.

The inhibitor slows or, in high concentration, virtually stops the growth of capillaries needed to feed the developing cancer tumor. It could be used to study diseases involving abnormal blood vessel growth, including diabetic and sickle cell retinopathy, arthritis, and psoriasis. The primary concern, however, is for understanding tumor growth.

Angiogenesis (AG) is the process through which tumors attract new blood vessels vital for continued growth. (See *Reporter*, December 1981.) AG inhibitors (AGI's), some of which are proteins with an unusually large number of positive charges, have been isolated from cartilage and the vitreous humor of the eye, but their limited quantities have stymied research.

Early experiments by Dr. Robert Langer, Massachusetts Institute of Technology, and Dr. Judah Folkman, Children's Hospital Medical Center, Boston, revealed the promise of AGI therapy. Using a drug-delivery carrier devised by Dr. Langer, experiments showed that AGI derived from cartilage inhibited mouse and rabbit tumor growth.

The new AGI protamine is an arginine-rich, positively charged protein found only in sperm. The discovery is significant because protamine's structure is known, and protamine is commercially available. While protamine should be of great importance to AG investigators, its use as a therapeutic agent is ruled out because it is cumulatively toxic in animals.

"The next question is to see if there are any compounds related to protamine that have the same desired effects without the toxicity," says Dr. Folkman. Andrus

Leaping salmon: *a protein derived from salmon sperm inhibits the growth of blood vessels that feed cancer tumors in laboratory animals.*

Photo by George B. Kelez, U.S. Fish and Wildlife Service

Professor of Pediatric Surgery at Harvard Medical School.

The discovery that mast cells, the formative cells of connective tissue, were associated with new blood vessels at a tumor site led to the protamine research, Dr. Folkman says.

"We found that the mast cells released heparin, which increased the ability of capillaries to move toward the tumor," Dr. Folkman says. "Stephanie Taylor, a Harvard Medical School student, found that heparin increased tumor-induced AG in chick embryos but that in the absence of tumor growth heparin alone has no effect on AG."

It is known that protamine neutralizes heparin, an anticoagulant. Tests showed that a small amount of protamine reduced AG and a larger amount stopped it completely. The reasons for protamine's AGI activities are not known.

The availability of protamine made it possible for Dr. Folkman's team to study the systemic administration of an AGI. Protamine's toxicity is noticeable in high doses, causing lethargy, weakness, and sometimes death in laboratory animals. The protein, however, did produce a 77 to 97 percent inhibition of the size of lung metastases in mice and inhibited the growth of a tissue culture strain of B16 melanoma injected subcutaneously in mice. No effect was found on two other types of tumors grown subcutaneously.

"Protamine can cause regression in some lung tumors because it is taken up by the lung in high concentrations," Dr. Folkman says. Conversely, skin uptake of protamine is low.

At present, Dr. Folkman and his colleagues are concentrating on finding the mechanism of AG. "We know that the nonanticoagulant part of the heparin molecule is apparently very important to the capillary endothelial cell response to an AG stimulant," he says. "We are trying to understand how protamine turns off the ability of the endothelial capillary sprouts to grow. When we know that mechanism, we might know how to identify other compounds that will do the same thing."

To that end, Dr. Folkman is working with cultured capillary endothelial cells to see what makes them grow toward tumor cells. "It's still premature," he adds, "to say whether the inhibition of AG will lead to a new therapy."

For further reading:

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Research into angiogenesis inhibitors by Dr. Folkman and Ms. Taylor is supported by the National Cancer Institute and a grant from the Monsanto Corporation to Harvard University. Pilot research by Drs. Langer and Folkman was supported by the National Institute of General Medical Sciences, the Biomedical Research Support Grant Program of the NIH Division of Research Resources, the American Cancer Society, the Juvenile Diabetes Foundation, the American Diabetes Association, the Monsanto Company, and the Alza Corporation.

Research Service

Monkey Colonies for Research on Aging

Colonies of aged monkeys at four NIH Regional Primate Research Centers (PRC's) have been set aside for research on the aging process.

A total of 187 aged macaques are available: 55 *Macaca mulatta* (rhesus monkeys) at the California PRC in Davis, birth years 1964 to 1967; 49 *M. mulatta* at the Oregon PRC in Beaverton, birth years 1961 to 1967; and 56 *M. mulatta* at the Wisconsin PRC in Madison, birth years 1956 to 1967; and 27 *M. nemestrina* (pigtailed macaques) at the Washington PRC in Seattle, birth years 1957 to 1967. Complete experimental and clinical histories of the animals are on record at the respective PRC's.

The primary use of the aged animals will be in experiments that will not cause them long-term or irreversible effects. Tissue specimens, organs, blood, skeletal structures, viral specimens, and other biological samples may be provided, when available, to NIH National Institute on Aging (NIA) grantees. Some services may also be provided to NIA grantees by the PRC's. Investigators should contact the director of the appropriate PRC and negotiate agreements prior to submitting a grant application involving use of these animals.

The set-aside colonies were created through an interagency agreement between NIA and the NIH Division of Research Resources. An independent panel of primatologists and comparative pathologists selected the monkeys for the set-aside colonies from a pool of 360 monkeys, 15 years old or older, on the basis of their experimental and clinical histories. Animals that have been breeders or have been exposed to short-acting drugs or to interventions with expected local or mild effects were selected.

In most cases, the aged animals will be maintained at the respective PRC's for the duration of the investigators' grants. Under rare circumstances a small number of monkeys may be purchased from the PRC's and transferred to other laboratories. Negotiations should be made with the director of the appropriate PRC and coordinated through the director of the Aging Primate Research Resource Program at the NIA.

For additional information about the set-aside colonies and other services contact:

Dr. Leonard Jakubczak
Building 31, Room 5C05
National Institute on Aging
Bethesda, Maryland 20205
Telephone (301) 496-3136

Report on Animal Models Of Diabetes Available

A report describing animal models used in the study of diabetes mellitus is available from the NIH Fogarty International Center. Entitled "Proceedings of a Task Force on Animals Appropriate for Studying Diabetes Mellitus and Its Complications," it covers models that can be utilized to examine problems in the etiology, pathogenesis, and underlying mechanisms of diabetes mellitus.

Fifty-seven experts in diabetes mellitus research or animal genetics contributed to the report as members of a task force convened by the NIH Diabetes Coordinating Committee. Their findings, compiled during a 4-month evaluation of biomedical literature, were published as a supplement to *Diabetes* in April, 1982.

A limited number of copies of the report are available on written request. Send a self-addressed label to Ms. Susan Stark, Attention: DIABETES, Publications Office, Fogarty International Center, Building 38A, Room 609, Bethesda, Maryland 20205.

Reprints of Reporter Articles Available

Reprints of several articles published in past issues of the *Research Resources Reporter* are available on written request and are listed below.

Since the quantities of these reprints are limited, requests will be filled on a first-come, first-serve basis. Requests for multiple copies of a single article should state how the copies will be used. Send requests to: Research Resources Information Center, 1776 East Jefferson Street, Rockville, Maryland 20852.

Reprints available, by title and *Reporter* month of issue, are:

Biomedical Computers Bridge Gap Between Technology and Researchers, March 1978.
GCRC Research Teams Study Link Between Psychosocial Dwarfism and Child Abuse, July 1978.
Research into Autism Yields New Insight, November 1978.
Physicians Search for Cause of Lyme Arthritis, January 1979.
Albany Unit Develops New Shock-Trauma Techniques, February 1979.
Powers Discusses Shock-Trauma Research, February 1979.
New Tests for Connective Tissue Diseases, February 1979.
Animal Resources Essential to Biomedical Research, August 1979.
Behavioral Therapy, April 1980.
Theory Explains Gout Attacks, November 1980.
Human Growth Hormone: Potentials for Treatment Are Broadened, December 1980.
Monoclonal Antibodies for Tracing the Processes of Tumor Formation in Neuroblastoma, July 1981.
Family Behavior: Key to Managing Juvenile Diabetes, August 1981.
Bone Loss in the Elderly: The Search for Causes of Osteoporosis, November 1981.
Research into Neuroendocrinology: The Study of Diverse Hormonal Influences, April 1982.
Diagnosis, Cause, and Treatment of Alzheimer's Disease, June 1982.

Who's Watching?

The following is a sample of bloopers found in site visit reports gathered recently at the General Clinical Research Centers Program of the NIH Division of Research Resources:

The nursing staff has been stable for several years.

The use of a joystick by the subject may cause stimulation of the motor cortex and remotor structures.

The dietitian is very capable and is involved with the investigators.

This is akin to a fishing exhibition.

Ontario Sclerosis.

An embarrassment of sickness of ideas.

By 2 weeks most patients have normalized their blood glucose levels and have used the pump for periods of 3 to 26 months.

The GCRC grant is a major leech pin in our clinical research efforts.

Unheirited abnormalities in growth.

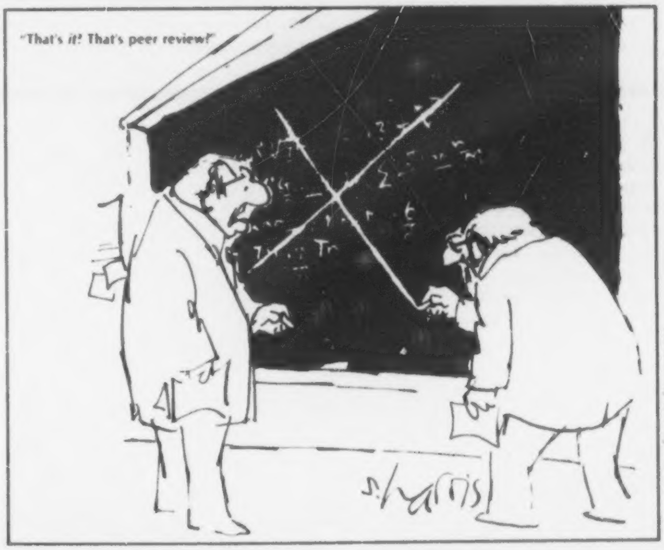
This Center seems to be unique in being one of the few.

Dr. D. administers yearly functions in the Core Laboratory.

Nursing turnover has been low and morals high.

As young wifes apply the method to man. . .

X-rays of the lateral feet.



"That's it! That's peer review!"

Cartoon by Sidney Harris © 1982

Meetings

September 20-23, 1982

Title: *Cardiovascular Medicine and Surgery in the 1980's*
Location: *Athens, Greece*
Contact: *Office of the Medical Director*
Texas Heart Institute
P.O. Box 20269
Houston, Texas 77225
Telephone (713) 791-2157

September 26-October 2, 1982

Title: *5th World Congress of Ultrasound in Medicine*
Location: *London, England*
Contact: *Secretariat*
4 "L" Portman Mansions
Chiltern Street
London, W1M 1LF England

September 27-October 1, 1982

Title: *1st International Congress of Neuroimmunology*
Location: *Stesa, Italy*
Contact: *Dr. J. Spreafico*
Mario Negri Pharmacological Research Institute
Via Eritrea 62
20157 Milano, Italy

October 1-2, 1982

Title: *Recent Research Advances in Aging, Heart Disease, Cancer, and Other Age-Related Disorders*
Location: *Los Angeles, California*
Contact: *Robert J. Morin, M.D.*
UCLA and American Longevity Association
1000 West Carson Street
Torrance, California 90509
Telephone (213) 533-2233

October 4-7, 1982

Title: *7th INSERM Colloquium on Hormones and Cell Regulation*
Location: *Alsace, France*
Contact: *Dr. R. Denton*
Department of Biochemistry
University of Bristol Medical School
Bristol BS8 1TD,
United Kingdom

October 4-8, 1982

Title: *American Institute of Ultrasound in Medicine/Society of Diagnostic Medical Sonographers Annual Convention*
Location: *Denver, Colorado*
Contact: *Barbara Hairfield*
Suite 504
4405 East-West Highway
Bethesda, Maryland 20814
Telephone (301) 656-6117

October 10-13, 1982

Title: *Annual Meeting of the American Academy of Neurological Surgery*
Location: *Boston, Massachusetts*
Contact: *J. T. Garner, M.D.*
1127 East Green Street
Pasadena, California 91106
Telephone (213) 793-2119

October 16-22, 1982

Title: *Joint American Society of Clinical Pathologists and College of American*

Pathologists Fall National Meeting
Location: *Miami Beach, Florida*
Contact: *Mr. Patrick E. Raleigh*
2100 West Harrison Street
Chicago, Illinois 60612
Telephone (312) 738-1336

October 17-22, 1982

Title: *International Congress of Allergology and Clinical Immunology*
Location: *London, England*
Contact: *R. W. Neal*
Suite 602
350 Sparks Street
Ottawa, Ontario
Canada K1R 7S8

October 17-22, 1982

Title: *10th World Congress of Gynecology and Obstetrics*
Location: *San Francisco*
Contact: *Judy Fielden*
American College of Obstetrics and Gynecologists
Suite 300E
600 Maryland Avenue, S.W.
Washington, D.C. 20024
Telephone (202) 638-5577

November 3-9, 1982

Title: *Lessons from Animal Diabetes: A Workshop*
Location: *Jerusalem, Israel*
Contact: *Professor Eliazar Shafrir*
Department of Biochemistry
Hadassah University Hospital
P.O. Box 12000
Jerusalem 91120, Israel

November 15-16, 1982

Title: *Birth Defects Institute Symposium 13: Recombinant DNA and Medical Genetics*
Location: *Albany, New York*
Contact: *Ian H. Porter, M.D.*
Director
Birth Defects Institute
New York State Department of Health
Room 2509
Rockefeller Empire State Plaza, Tower
Albany, New York 12237
Telephone (518) 474-1929

December 2-4, 1982

Title: *2nd Perinatal Intracranial Hemorrhage Conference*
Location: *Washington, D.C.*
Contact: *Jerold F. Lucey, M.D.*
Chief of Newborn Services
Shep 6-MFU
Medical Center Hospital of Vermont
Burlington, Vermont 05401
Telephone (802) 656-4276

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This Issue:

Dr. Betty H. Pickett
Appointed Director of
Division of Research Resources
See page 3.

By
Gail Shiner Makulowich

Perinatal Center Improves Diagnosis of Intrauterine Growth Retardation

Growth retardation of the fetus in the womb often goes undetected by doctors until birth. These small, fragile babies—usually defined as weighing less than 90 percent of the average weight of babies of the same gestational age—comprise 10 percent of all births. They are more at risk for intrauterine and neonatal death, asphyxia during labor, and long-term neurological disability than babies who are the appropriate size for gestational age (AGA).

If it is suspected that a woman is carrying a baby smaller than it should be for the estimated due date, an ultrasound examination, or sonogram, allows measurement of the fetal head size to determine whether the baby is smaller than expected. However, factors that determine due dates are often unreliable. Therefore, it is difficult to distinguish between the small fetus that is appropriately grown for gestational age but less mature than the due date suggests and the fetus that is appropriately mature for the due

16th century woodcut of a woman in labor as an astrologer looks to the stars to predict the outcome of the delivery: ***today, sophisticated diagnostic tests are used to predict the unborn infant's state of health.***

HE 20.3013/6:

IUGR *continued*

Dr. Robert Sokol, Mr. Thomas Williams, and Dr. Thomas Gross (left to right) examine computer data: *developing a massive computerized perinatal data base for correlating maternal and fetal factors with fetal health.*

date but inappropriately small for gestational age (SGA)—that is, growth retarded.

A reliable measurement of fetal maturity must be combined with measurements of fetal size to make this distinction, according to Dr. Robert J. Sokol, professor of obstetrics and gynecology and director of the Perinatal Clinical Research Center at the Cleveland Metropolitan General Hospital/Case Western Reserve University in Ohio. He and a diverse group of researchers have been studying intrauterine growth retardation (IUGR) at the center for 6 years. They have developed a data base on 25,000 consecutively delivered women, probably the largest computerized perinatal data base in the United States, and are currently using it to assess the reliability of clinical risk information, fetal maturity tests, and other laboratory tests in the diagnosis of IUGR before birth. Their goal is to develop a diagnostic model that will enable clinicians to detect far more cases of IUGR than the 20 percent Dr. Sokol estimates are predicted with currently available clinical methods.

The increased ability to diagnose IUGR prenatally will make it possible for obstetricians to improve the health of babies with some types of growth retardation, particularly those whose growth becomes slowed in the third trimester, when most of the gross body weight accumulates, says Dr. Sokol. Growth-retarded babies are a heterogeneous group; the cause of growth alteration determines the developmental stage at which the fetus is affected. Major known causes, often called risk indicators for IUGR, include genetic abnormalities; maternal medical complications such as chronic hypertension, preeclampsia, kidney disease, and substance abuse; intrauterine infections such as rubella, toxoplasmosis, and cytomegalovirus disease; maternal malnutrition; and constitutionally small, very lightweight mothers, who sometimes have growth-retarded babies.

Babies with congenital anomalies tend to be the most severely growth retarded and can exhibit poor growth as early as the first trimester of pregnancy, says Dr. Sokol. Intrauterine infection and abuse of cigarettes, alcohol, and other drugs can affect growth throughout pregnancy. Intrauterine infections during the first trimester tend to reduce the number of fetal brain cells and other cells; problems later in pregnancy restrict growth in the size of cells. Babies affected by the earlier intrauterine infections, therefore, tend to have more severe, less treatable neurological problems because the brain has an inadequate number of cells. It is not clear when substance abuse might have the most serious impact on fetal growth and well-being, says Dr. Sokol. For example, animal studies show that maternal alcoholism during the first trimester causes congenital anomalies in offspring. Some human studies, however, show that when drinking is reduced or stopped in later pregnancy, there is a decreased incidence of congenital anomalies, indicating that the most damage from alcohol may occur after the first trimester. Chronic hypertension, preeclampsia, kidney disease, and maternal malnutrition tend to restrict the flow of blood and nutrients to the placenta and fetus, retarding the growth of both. The fetus whose growth is ultimately retarded because of these problems or unknown causes tends to grow normally until the third trimester, according to Dr. Sokol. Then the growth curve flattens.

"There seems to be a correlation between the duration and severity of IUGR and the risk of later abnormalities, although the association is still being actively investigated," says Dr. Sokol. "It's probably true that the longer growth retardation persists in the womb, the more severe the neurobehavioral problems, such as mental retardation and cerebral palsy. We have the best opportunity to improve the outcome of small babies whose growth becomes slowed in the third trimester, because they tend to have less serious problems and a higher chance of developing normally after birth."

But the currently limited repertoire of available clinical methods makes prenatal detection of IUGR very difficult, according to Dr. Sokol. The clinician usually estimates gestational age on the basis of maternal menstrual history, first audible fetal heart tones, first fetal movement felt by the mother, progression of uterine growth, and maternal weight gain. These indicators of fetal age are often unreliable.

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And frequently a physician does not see a pregnant patient until too late in the pregnancy to adequately document them, so there is often no reliable way to correlate fetal age with size.

Although clinical factors can alert the physician to suspect IUGR, they are not sufficient to confirm a diagnosis of IUGR, asserts Dr. Sokol. Over the past 6 years, he and his colleagues have performed an extensive study to assess their ability to predict growth retardation before birth, based on clinical methods alone. On the basis of data collected over the past 10 months they suspected IUGR about one-third of the time and correctly predicted about one-half of the cases. "While this is a little more accurate than the 20 percent of cases most doctors correctly suspect, the study demonstrated that the ability of clinical data alone to predict IUGR is very limited," concluded Dr. Sokol.

During the past several years, Dr. Sokol and his colleagues have developed a group of laboratory diagnostic procedures ranging from amniocentesis and sonogram measurements to glucose tolerance tests and maternal metabolic profiles. They will soon test them in varying combinations to determine which set of tests best detects IUGR. This set of procedures will comprise the clinical model for diagnosing IUGR.

Initially, the investigators studied amniotic fluid indicators of fetal lung maturity—lecithin-sphingomyelin ratio, number of fat cells, and level of creatinine—to determine if they could distinguish whether a baby was small because of miscalculated gestational period or because of growth retardation. To accomplish this they correlated markers of lung maturity with biparietal diameter (BPD) of the fetal head. Despite sophisticated computer analysis, the differentiation of appropriate growth and growth retardation was not accurate enough, according to Dr. Sokol. In another study the researchers developed a good method for detecting amniotic fluid phosphatidylglycerol (AF PG). They

Dr. Pickett Becomes DRR Director

Dr. Betty H. Pickett

Dr. Betty H. Pickett became the director of the NIH Division of Research Resources (DRR) on October 1, 1982. Dr. Pickett, former deputy director of the National Institute of Child Health and Human Development (NICHD), was appointed to the position by NIH director Dr. James B. Wyngaarden.

As the director of DRR, Dr. Pickett will preside over a broad range of research centers, resources, and institutional support programs. The five DRR programs include clinical research centers, biotechnology resources, animal resources, minority biomedical research support, and biomedical research support.

Dr. Pickett, deputy director of NICHD since 1979, served concurrently as acting director of that institute from July 1981 to July 1982. Her work at NICHD followed 1½ years as associate director for the Extramural and Collaborative Research Program of the National Institute on Aging.

From 1963 to 1977, Dr. Pickett served in a number of senior positions at the National Institute of Mental Health, including 3 years as the executive secretary of the Research Career Program; 1½ years as the chief of the Cognition and Learning Section, Behavioral Sciences Research Branch, Division of Extramural Research Programs; 6½ years as deputy director of the Division of Extramural Research Programs; 1 year as director of the Division of Special Mental Health Programs; and 2 years as acting director of the Division of Extramural Research Programs.

Since 1970 Dr. Pickett has served on 17 NIH or NIMH task forces, committees, panels, or advisory groups. Among these were membership from 1974 to 1977 on the NIMH Director's Research Advisory Group and, in 1980, on the NIH Alcohol, Drug Abuse, and Mental Health Administration Interagency Committee on Health and Behavior.

Dr. Pickett has received numerous honors and awards, including the Department of Health, Education, and Welfare Distinguished Service Award in 1975, the FEW Award for Distinguished Service in Support of Equal Opportunities for Women in 1975, the Brown University citation for distinguished public service, the Harold M. Hildreth Award of the American Psychological Association for distinguished contribution to psychology in the public service in 1979, and the Senior Executive Service Award for Outstanding Performance in 1981.

A native of Rhode Island, Dr. Pickett received a bachelor of arts degree magna cum laude from Brown University in 1945 and was inducted that year into Phi Beta Kappa and Sigma Xi. She continued at Brown University, earning a Ph.D. in psychology in 1949.

She is a member of eight professional societies: American Psychological Association, Eastern Psychological Association, Psychonomic Society, Association of Women in Science, American Association for the Advancement of Science, Society for Research in Child Development, Academy of Behavioral Medicine, and Sigma Delta Omicron for Graduate Women in Science.

IUGR *continued*

found the presence of AF PG to be the best indicator of fetal lung maturity.

Along with lecithin, AF PG is a major component of surfactant, a substance that is produced by the fetal lung to reduce surface tension, enabling the air sacs to remain expanded after birth. Babies usually do not have sufficient surfactant to breathe properly on their own until near term, and it is hypothesized that AF PG abruptly appears close to term to stabilize the surfactant. "While there is a strong correlation between fetal lung maturity and gestational maturity, they are not synonymous," says Dr. Thomas L. Gross, assistant professor of obstetrics and gynecology, Cleveland Metropolitan General Hospital/Case Western Reserve University. "Researchers have used AF PG for the last few years as a measure of fetal lung maturity. Since it appears in amniotic fluid close to term, we decided to use it as a marker of term gestation, and thus fetal age, and to correlate it with fetal size to see if we could predict intrauterine growth retardation."

In a preliminary study of 82 pregnancies that yielded infants of low birth weight—less than 2,700 grams—PG was the only amniotic fluid phospholipid that correctly predicted SGA or growth-retarded infants, says Dr. Gross.

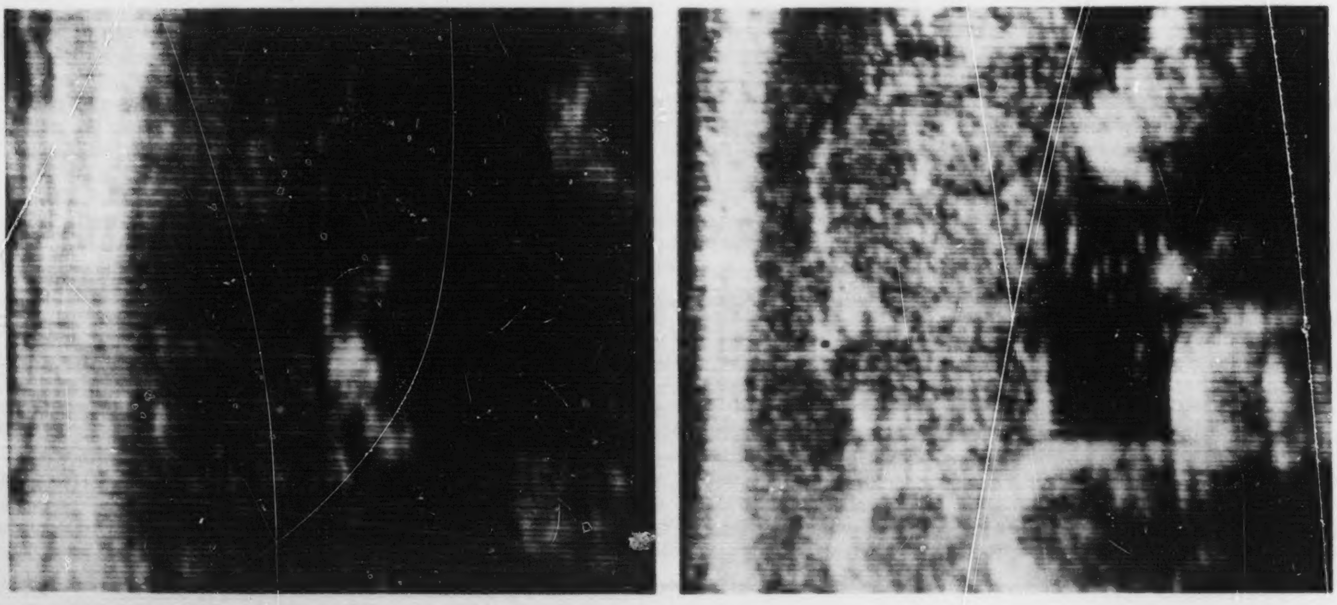
In a recent followup study, Drs. Sokol and Gross and their colleagues used ultrasonography to measure the BPD of the fetal head at or beyond 34 weeks of gestation and within 1 week of delivery, to screen out babies that were not small, that is, those with a BPD of 87 mm or more. On clinical indication, they performed amniocentesis on the women with small babies, to determine the amniotic fluid PG levels. Using sonographic screening and the AF PG test in 249 pregnancies, the researchers were able to detect IUGR in 80 percent of the growth-retarded babies. Moreover, a positive AF PG test following sonographic indication of a small fetus was associated with a fifteenfold greater risk of delivering a growth-retarded infant than was a negative AF PG test.

Because PG usually appears abruptly in the amniotic fluid close to term it is rarely helpful in detecting IUGR until late in the third trimester of pregnancy. Also, amniocentesis requires a sufficient amount of amniotic fluid in the womb to provide safe withdrawal of a sample. Some women carrying growth-retarded babies may lack adequate amniotic fluid, a condition called oligohydramnios, which increases the risk that the needle inserted into the mother's uterus to withdraw the fluid may strike the fetus.

In pursuit of less invasive techniques that could be performed earlier in pregnancy, the researchers explored other fetal maturity indicators such as placental aging. Placentologists have been studying the relationship between placental changes and fetal outcome for a long time. Recent advances in ultrasound that permit visualization of the placenta in situ have enabled researchers to document progressive changes in the placenta as pregnancy advances. A measure of placental aging, called placental grading, was developed in 1979. Grading ranges from zero in early pregnancy, when the placenta is homogeneous with no calcification, to grade 3 in late pregnancy, when the placenta becomes calcified and fibrotic.

Other researchers had previously correlated a grade 3 placenta with fetal lung maturity, but no one had correlated it with growth retardation, according to Dr. George Kazzi, assistant professor of reproductive biology and clinical research fellow in maternal-fetal medicine at Cleveland Metropolitan General Hospital/Case Western Reserve University. He and Drs. Gross and Sokol retrospectively evaluated the potential of the grade 3 placenta to predict growth retardation in 109 pregnancies that resulted in babies weighing 2,700 grams or less. The results showed that for the small fetus a grade 3 placenta prior to 34 weeks gestation is associated with a fourfold increase in risk for IUGR, compared to pregnancies in which placental grade is less than 3 at that time. Sonographic documentation of fetal maturity by grade 3 placenta allowed the re-

Sonographic cross sections of homogeneous grade 1 placenta (left) and calcified grade 3 placenta: *correlating the calcification of the placenta and the length of pregnancy with fetal growth retardation.*



searchers in this study to correctly classify the small fetus as preterm or growth retarded and to detect growth retardation in 65 percent of the growth-retarded babies. In a followup prospective study of pregnancies that resulted in babies who were LGA (large for gestational age), SGA, and of normal size, grade 3 placenta prior to term was associated with a threefold to fourfold increase in risk for IUGR.

"The placenta naturally ages as it gets closer to term," says Dr. Kazzi. "Rarely will you see a grade 3 placenta before 34 weeks of gestation in normal pregnancies. In our study, however, there were a few patients in whom the grade 3 placenta appeared as early as 28 or 29 weeks of gestation. So our findings indicate that the placenta ages earlier in pregnancies in which the fetus is stressed or growth retarded."

It is not surprising that the placenta undergoes changes in the presence of intrauterine stress, adds Dr. Kazzi. Like any other fetal organ, it is subjected to the same stress mechanisms that are believed to result in altered fetal growth. For example, pregnancies complicated by chronic hypertension usually result in vascular lesions in the placenta that interrupt the oxygen and nutrient supply to the fetus.

Prior to the use of ultrasound, clinicians were able to examine the placenta for pathological changes only after birth of the baby. Similarly, they were able to reliably detect oligohydramnios only when the amniotic sac broke during labor and the volume of amniotic fluid was slight. Now oligohydramnios can also be detected by ultrasound.

The association of oligohydramnios, at-risk pregnancy, and retarded fetal growth has been recognized for a long time, according to Dr. Sokol. Exploring the potential of ultrasonic measurement of oligohydramnios to detect IUGR, Dr. Elliot Philipson, a perinatal center investigator, Dr. Sokol, and their colleagues used the center's computerized data base to evaluate the relationship of decreased amniotic fluid volume to SGA birth. "The results suggested that if oligohydramnios appears on ultrasound examination, there's a 50-50 chance of having a growth-retarded baby," says Dr. Sokol. "But," he cautions, "oligohydramnios occurs very infrequently, in only about 4 percent of our patients. Many times, oligohydramnios is not present with a growth-retarded baby, so by itself, oligohydramnios is not a very good predictor of IUGR. A second research study of ours did show, however, that if we assessed sonographic measurement of oligohydramnios in combination with certain clinical factors, it did improve our ability to predict IUGR."

Other studies at the perinatal center are exploring the potential for predicting IUGR by observing maternal metabolic changes during pregnancy. Maternal metabolism affects the amounts and types of nutrients available to the fetus. Clinicians have long attributed the unusually large size of babies of diabetic mothers to the excessive amounts of glucose and other substrates that cross the placenta from mother to fetus. Dr. Satish C. Kalhan, director of the pediatric metabolism unit, Cleveland Metropolitan General Hospital, Dr. Sokol, and their colleagues hypothesized that reduced availability of glucose to the fetus might result in babies who are small for gestational age. To explore this theory they gave 55 pregnant mothers an intravenous

Research nurse coordinator Renee Dunnington examines a low birthweight infant in the neonatal intensive care unit: ***comprising 10 percent of all births, growth retarded babies are more at risk for intrauterine and neonatal death and long-term neurological disability than normal-sized babies.***

IUGR *continued*

glucose tolerance test during the third trimester of pregnancy. The results indicated that circulating levels of glucose in the mothers who delivered growth-retarded babies were lower than normal. "When maternal glucose levels are low, the amount of glucose delivered to the baby is likely to be less," explains Dr. Kalhan. "Our test indicates that just as diabetic pregnant mothers with hyperglycemia tend to have unusually large babies, mothers with low blood glucose levels may be more at risk of having growth-retarded babies. That's what the study implies, but we want to test it in a larger population. Although some mothers of growth-retarded babies appear to have lower levels of blood glucose, we don't know whether that's cause or effect. For example, there may be something wrong with the fetoplacental unit that prevents it from inducing the normal changes in blood glucose. More research is needed on this."

In the meantime, Drs. Kazzi and Kalhan and their colleagues have begun a study to correlate a variety of maternal metabolic changes during pregnancy with the outcome. They specifically want to compare changes in blood concentrations of glucose, amino acids, and fatty acids in mothers of normal-sized babies with these metabolic changes in mothers of growth-retarded babies. While not much fat is delivered to the fetus, glucose and amino acids are major sources of fetal energy and are needed for fetal tissue growth. "By correlating maternal differences in metabolism of these substrates with the weights of babies, we may find maternal metabolic changes that are associated with IUGR," says Dr. Kalhan.

When ongoing studies are completed, the research team will determine which combination of diagnostic tests—maternal metabolic profile, sonographic measurement of the fetus, amniotic fluid volume, placental grade, and level of amniotic fluid phosphatidylglycerol—together with clinical risk indicators will yield the most accurate detection of IUGR, that is, the best clinical model. "The model is intended to mimic the clinical decisionmaking process," explains Dr. Sokol. "A doctor rarely makes a decision based on one factor. He or she evaluates laboratory tests, physical examination, and patient history to arrive at an appropriate diagnosis. We use the computer to mimic that clinical decision-making routine, which is a series of deductive steps that takes new information into account. We want to refine the model so that it is sufficiently straightforward, noninvasive, simple, and inexpensive to be effectively used in clinical care."

Prediction of IUGR will enable clinicians to initiate improved care during pregnancy and to more safely manage labor and delivery. While little prenatal treatment is available for IUGR, some interventions are being tried. Hospital bed rest, for example, often improves the mother's nutritional intake and enables avoidance of substance abuse, if these are contributing factors, and it may improve overall blood flow to the uterus. A number of researchers have also used hypertonic glucose to increase the mother's glucose load, thereby increasing fetal glucose intake. While the data on these interventions are not convincing there may be some cases of growth retardation that respond to these treatments, says Dr. Sokol.

A more important immediate benefit of IUGR diagnosis prior to birth is that it does alert the doctor to an at-risk birth, so that appropriate fetal monitoring can be done and specialized staff and facilities can be prepared for labor and delivery of these fragile babies. IUGR diagnosis may also alert the doctor to monitor factors of fetal well-being more frequently prior to labor; for example, periodic fetal heart monitoring and frequent analysis of maternal blood estriol levels, an indicator of placental sufficiency. Labor management, however, cannot improve the outcome of growth-retarded infants affected by certain congenital anomalies, cautions Dr. Sokol. Babies with Down syndrome, for instance, will be retarded regardless of how the labor is managed. But careful labor management can significantly improve the outcome of the large group of babies whose growth was slowed in the third trimester by any of a variety of causes. "Overall, I would speculate that 50 to 70 percent of growth-retarded babies have the potential to grow and develop normally, particularly if detection of the problem before birth enables us to properly manage labor and delivery," concludes Dr. Sokol.

Additional reading:

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2. Gross, T. L., Sokol, R. J., Wilson, M. V., and Zador, I. E., Using ultrasound and amniotic fluid determinations to diagnose intrauterine growth retardation before birth: a clinical model. *American Journal of Obstetrics and Gynecology* 143:265, 1982.
3. Gross, T. L., Sokol, R. J., Wilson, M. V., et al., Amniotic fluid phosphatidylglycerol: a potentially useful predictor of intrauterine growth retardation. *American Journal of Obstetrics and Gynecology* 140:277, 1981.
4. Sokol, R. J., Kazzi, G. M., Kalhan, S. C., et al., Identifying the pregnancy at risk for intrauterine growth retardation: possible usefulness of the intravenous glucose tolerance test. *American Journal of Obstetrics and Gynecology* 143:220, 1982.
5. Philipson, E. H., Sokol, R. J., and Williams, T., Oligohydramnios: clinical associations and predictive value for intrauterine growth retardation. *American Journal of Obstetrics and Gynecology*. Submitted for publication.
6. Petrucha, R. A., Golde, S. H., and Platt, L. D., Real-time ultrasound of the placenta in assessment of fetal pulmonary maturity. *American Journal of Obstetrics and Gynecology* 142:463, 1982.
7. Ounsted, M., Moar, V., and Scott, W. A., Perinatal morbidity and mortality in small-for-dates babies: the relative importance of some maternal factors. *Early Human Development* 5:367-375, 1981.
8. Davies, D. P., Growth of small-for-dates babies. *Early Human Development* 5:95-105, 1981.

Intrauterine growth retardation research conducted at the Perinatal Research Center is supported by the National Institute of Child Health and Human Development, the National Institute on Alcohol Abuse and Alcoholism, and the General Clinical Research Centers Program of the NIH Division of Research Resources.

Research Focus

Progress in Diabetes Research

Last year marked the 20th anniversary of the General Clinical Research Centers (GCRC) Program, which was established to study and resolve significant health problems in the United States. This summary of GCRC and related research is based on a report submitted to the program by the Advisory Group on GCRC Advances in Diabetes, chaired by Dr. Bernard M. Babior, professor of medicine at Tufts-New England Medical Center in Boston.

Physicians may now be close to delaying or preventing many long-term complications of diabetes mellitus, a disease that affects about 1 of every 100 Americans, according to the report. Complications associated with diabetes, which the report claims is the third leading cause of death in the United States, include atherosclerosis, retinopathy, and nephropathy.

In type I diabetics—those with the most serious complications—the insulin deficiency is virtually complete, leading to highly unstable blood sugar levels and a lifelong need for insulin injections. GCRC's have provided evidence of hereditary factors that predispose a person to type I diabetes. And they have demonstrated the presence of cytotoxic anti-islet cell antibody in the serum of these patients.

Persons genetically predisposed to the disease may acquire a viral infection of the beta cells of the pancreatic islets, rendering them susceptible to destruction through an autoimmune process. The suggestion of a viral etiology raises the possibility of developing a vaccine.

Investigators at the University of Minnesota GCRC believe the inherited predisposition is genetically heterogeneous. At least two diabetic-response genes are suspected. One is associated with the human leukocyte antigen (HLA) system, which is partly responsible for the body's ability to recognize foreign substances. It is believed that this gene transmits the predisposition as a recessive trait. The other gene is not associated with HLA. It may transmit the predisposition as a dominant trait.

Researchers at the Children's Hospital of Philadelphia GCRC, however, believe that a single HLA-linked gene transmits the predisposition, which then leads to a modest susceptibility in the heterozygote and to a pronounced vulnerability in the homozygote. In studying evidence of an autoimmune process related to diabetes, Duke University GCRC investigators discovered an antibody that was cytotoxic to cultured rat beta cells in the sera of 8 of 24 type I diabetics. Cytotoxicity depended on the presence of complement. The antibody was absent from the sera of 34 normal patients and 2 patients with polyglandular failure but was present in 1 of 12 serum samples from type II diabetics or those with maturity-onset diabetes. Type II diabetics, comprising

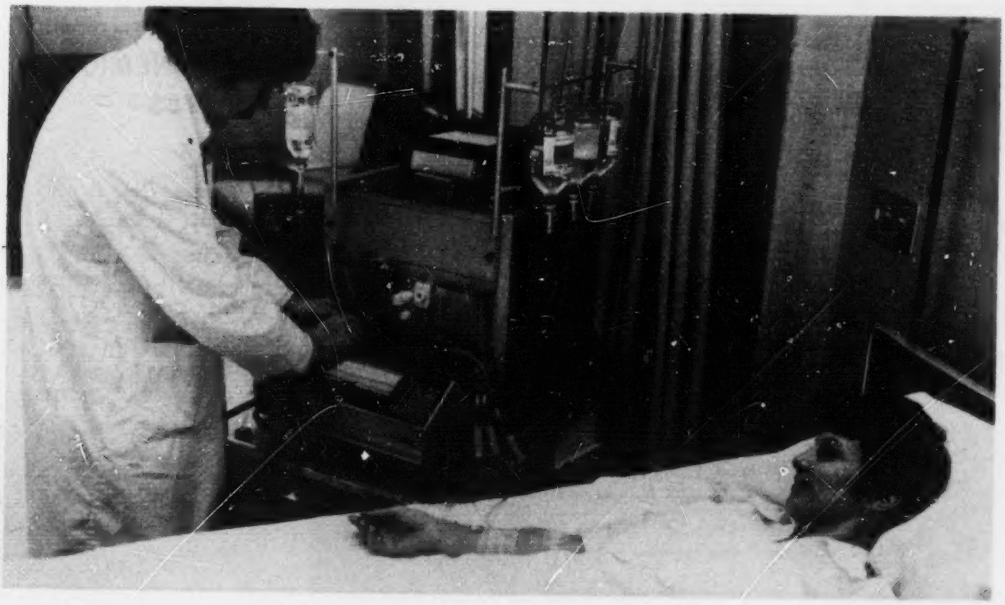


Photo courtesy of the Mayo Clinic

The Biostator, a mechanical pancreas that provides insulin and regulates the body's glucose level, being tested at the Mayo Foundation GCRC: ***improving control of blood sugar with biomedical devices.***

about 80 percent of those with the disease, produce some insulin, but the condition is aggravated by a decrease in tissue sensitivity to the hormone.

Research on obese, nondiabetic individuals at several GCRC's has helped investigators understand some of the problems faced by diabetics. Investigators observed that basal insulin levels were elevated in obese individuals. The secretion pattern matched that of lean persons, however, when the levels were expressed as a percentage of basal activity. Such basal insulin levels were used to disprove the hypothesis that maturity-onset diabetics secreted excessive insulin. Using basal insulin levels, researchers found that the apparent hyperinsulinism in obese diabetics was due to their greater body weight and that insulin responses to glucose in all diabetics were abnormally low.

These were among the first findings to establish that the ability of beta cells to respond to glucose is deranged in diabetics. Work at the Stanford University GCRC showed that changes in plasma insulin levels were due to alteration in the amount of insulin secreted rather than to change in the rate of metabolic clearance of the hormone. A two-phase dynamic model of insulin release, developed at the University of Washington in Seattle, identified first- and second-phase releases of insulin. The first or acute phase was found to be defective in type II diabetics. Apparently this phase depends on the fasting plasma glucose levels. At levels of plasma glucose below 115 to 130 mg/dl, insulin is released in a relatively normal manner. Above this level, first-phase release is attenuated. The first phase is also impaired in conjunction with lessening carbohydrate tolerance in healthy, older individuals. Experiments have shown that the first-phase defect is due to an impaired glucose receptor on the beta cell.

Insulin resistance also characterizes obese, nondiabetic individuals, according to findings at the University of Colorado and Stanford University GCRC's. The magnitude of resistance appears to be partially related to the patient's weight. Insulin resistance apparently represents an impediment to carbohydrate metabolism that can be overcome if the obese person has enough beta cell reserve to secrete excessive amounts of insulin.

The resulting hyperinsulinemia may have adverse metabolic effects such as promotion of hypertriglyceridemia, increased low-density lipoprotein (LDL) receptors, and LDL catabolism, with possible promotion of atherogenesis. Weight reduction can ameliorate most, if not all, of these abnormalities. The loss of as little as 20 pounds reduces insulin resistance, improves performance on glucose tolerance tests, and ameliorates hyperinsulinemia and hypertriglyceridemia. These benefits last as long as the weight loss is maintained.

Insulin resistance in obesity is due to heterogeneous mechanisms defined in several GCRC's. Isolated circulating monocytes and erythrocytes and isolated adipocytes are useful models in the study of insulin receptor function in normal and disease states. The isolated adipocyte, or fat cell, is biologically responsive to insulin and can be used to measure insulin-mediated glucose transport.

These cells have been used to determine that insulin receptors are decreased on a variety of target tissues from obese individuals. The magnitude of the decrease of receptors correlates well to the degree of hyperinsulinemia. This supports the theory that insulin can "down regulate" its own receptors—the higher the plasma insulin level, the lower the number of insulin receptors, and vice versa. In mildly insulin-resistant hyperinsulinemic patients, the insulin resistance is entirely due to a decrease in receptors. In the most severely resistant, a marked postreceptor defect in insulin action exists, although there are also receptor defects. Postreceptor defects, which are the predominant abnormality in most patients, are caused by a decrease in the intrinsic activity in the glucose transport system. Weight loss can rectify these problems.

Work from several GCRC's, including the one at Yale University, has demonstrated that most type II patients are resistant to the action of insulin in vivo whether they are obese or not. A decrease in insulin receptors is the primary cause of insulin resistance in patients with mild type II diabetes. Patients with fasting hyperglycemia also have a postreceptor defect that appears to be the predominant abnormality. Intensive insulinization with normalization of blood glucose concentrations for at least 2 weeks results in partial or

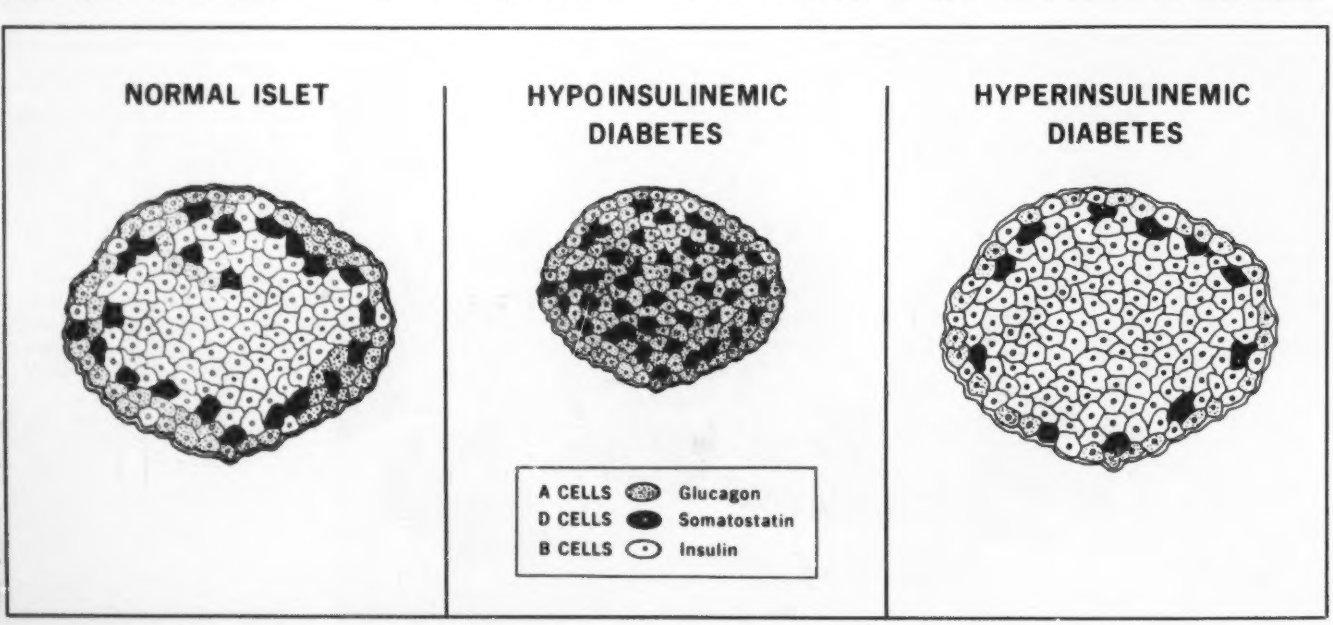


Photo reproduced with permission of *Diabetes* complete amelioration of the insulin-resistant state. This suggests that in type II diabetics, insulin deficiency occurs first, leading to an acquired form of insulin resistance that can be reversed.

Weight reduction in the obese diabetic improves control of hyperglycemia. It also increases the number of insulin receptors and reduces overall insulin resistance.

Drugs can also help in the treatment of obese and nonobese diabetics. Oral sulfonylurea agents strikingly increase the number of insulin receptors. Patients treated for up to several months have a 20 to 50 percent increase in insulin binding that is associated with an amelioration of overall in vivo insulin resistance and contributes to the efficacy of this class of drugs. These drugs also augment insulin secretion but modify the hormone's action. Aspirin has a beneficial effect on glucose control in some patients with maturity-onset diabetes. Salicylates raise serum insulin levels in normal persons and in type II patients by interfering with the synthesis of prostaglandins, which inhibit the release of insulin.

Prior to the development in 1960 of the immunoassay that allows the precise measurement of insulin in plasma, it was widely believed that glucose was the primary, if not the sole, signal to the pancreatic beta cells to release insulin. Research from 1966 to 1973 at the University of Washington revealed the major role of the autonomic nervous system in modulating insulin secretion. Experiments since 1973 have shown that several neurotransmitters, including acetylcholine, serotonin, and a variety of catecholamines and neuropeptides, influence the release of glucoregulatory hormones.

In 1966, University of Washington investigators found that epinephrine and norepinephrine given intravenously suppressed basal and glucose-induced insulin secretion. The inhibitory activity of catecholamines was shown to be mediated through alpha-adrenergic receptors. In ensuing experiments it was established that the control of insulin secretion via the sympathetic nervous system was a complex process involving effects on various catecholaminergic receptors. For example, isoproterenol, a beta agonist, was found to enhance rather than suppress insulin release. These tests revealed the interplay of tonic signals operating through different receptors on the islet cell to maintain homeostasis.

Research at the Albert Einstein College of Medicine and the Mayo Foundation GCRC's has shown that catecholamines, along with glucagon, are the most important of the counter-regulatory hormones. They cause acute changes in plasma glucose concentration, leading to instability and poor control in diabetic patients.

Following research at the University of Texas in Dallas and the Medical College of Wisconsin GCRC's, the prominent role of glucagon in the genesis of hyperglycemia was recognized. Circulating glucagon levels are usually suppressed by glucose or insulin infusions in normal subjects but not in diabetics. Tests have shown that circulating plasma glucagon levels are elevated in patients with all forms of diabetes, especially in those with type I diabetes that is poorly controlled. This condition probably contributes to hyperglycemia.

Researchers at Yale University and Vanderbilt University GCRC's have found that glucagon's major action is on hepatic glucose production, which the hormone promotes in several ways, including stimulation of glycogenolysis as well as promotion of gluconeogenesis.

With these concepts in mind, researchers at the University of Texas have proposed a bihormonal scheme for generation of hyperglycemia. According to this model, insulin deficiency is still the primary defect leading to hyperglycemia. After the onset of insulin deficiency, plasma glucagon levels rise, promoting hyperglycemia. Glucagon is then more difficult to suppress, which aggravates the situation.

Investigators at Vanderbilt University have also shown that glucagon is a potent ketogenic agent that contributes to diabetic ketoacidosis when profound insulin deficiency exists. Somatostatin has been important in demonstrating the role of glucagon in these situations. Researchers at several GCRC's have observed that when glucagon secretion is inhibited by somatostatin a reduction of hyperglycemia occurs. Thus, specific somatostatin-like analogs, which uniquely inhibit glucagon secretion, may be of therapeutic importance. When insulin is withdrawn from type I diabetics and glucagon secretion is inhibited by an infusion of somatostatin, the development of

Distribution and frequency of A, D, and B cells in the islets of Langerhans in the pancreas.

A lightweight, portable insulin infusion pump: ***freeing the diabetic from insulin injections while better controlling blood sugar.***

Photo courtesy of Cardiac Pacemakers, Inc.

hyperglycemic ketosis is delayed.

While the primary physiological abnormality of diabetes is hyperglycemia, this condition may not be responsible for the disease's most life-threatening feature—macrovascular changes. Atherosclerosis is the leading cause of death among diabetics, according to the report, with coronary artery disease accounting for more than one-half of all diabetic mortality.

Despite the advent of insulin treatment for diabetic hyperglycemia in 1926, the risk of death from atherosclerosis remains high, particularly in young patients. Because epidemiological data indicate that macrovascular changes may not be readily attributable to hyperglycemia, extensive investigations of lipid metabolism have been undertaken at the University of Cincinnati GCRC.

According to other Cincinnati studies, diabetics may have a particularly dangerous type of defect in lipid metabolism—elevations in serum cholesterol and triglycerides and a reduction in high-density lipoprotein (HDL). There is abundant evidence that prolonged exposure to this abnormality imposes a highly significant risk for coronary artery disease, even in nondiabetics. The superimposition of diabetes, however, results in a striking aggravation of atherogenesis. One study showed that elevated concentrations of LDL had a pronounced correlation with diabetic atherosclerosis. Similarly, a highly significant negative relationship of HDL with vascular disease was observed with varying specificity for differing diabetic subgroups. Thus, correction of this dyslipoproteinemia of elevated LDL and reduced HDL might represent a major focus of therapy.

Teams at the GCRC's of Stanford and Vanderbilt, the University of Washington, and Johns Hopkins University have undertaken studies to examine the mechanism of hyperlipemia in diabetes and to exclude an independent role for obesity and genetic predisposition. Researchers at Stanford and the University of New Mexico have found that insulin exerts a major influence on the triglyceride metabolism. Insulin increases hepatic production of lipoprotein and increases its removal via activation of lipoprotein lipase. Elevation in serum insulin can be correlated with increased hepatic production of very low-density lipoprotein (VLDL) triglyceride and with basal serum triglyceride concentration. Inhibition of insulin secretion with diazoxide results in an acute lowering of plasma triglyceride and VLDL.

Work at the University of Utah, the Medical College of Wisconsin, and the Universities of Washington, Cincinnati, and New Mexico GCRC's has established that estrogen also has a major influence on lipid regulation. The hormone appeared to act selectively on the liver to enhance production of VLDL triglyceride in a way similar to insulin. The hormone also reduced aminogenic glucagon secretion in much the same way as does ingestion of high-carbohydrate diets or human obesity. Progesterone has been shown to promote peripheral lipid removal via activation of lipoprotein lipase in an insulinlike manner. This research is important for female diabetics who take oral contraceptives, which contain progesterone and estrogen, or who are treated with these hormones following the onset of menopause.

Many of the investigations of lipid metabolism were initiated at a time when only triglyceride and cholesterol were believed to be important to lipid metabolism. Recently, however, GCRC researchers at the Universities of Cincinnati and New Mexico have postulated an important potential role for HDL in the prevention of atherogenesis. While HDL is recognized to have the capacity to remove free cholesterol from the cells of an atheroma, or mass of plaque, the limited amounts of HDL circulating in the diabetic may preclude successful prevention of cholesterol accumulation by an atheroma. One study showed an inverse correlation between plasma concentrations of HDL and the amount of cholesterol in human tissue. In the diabetic, irreversible atheroma formation can thus be visualized as a progressive abnormality induced by prolonged exposure to excess LDL and limited HDL, as in the aging process.

Possible therapies for problems of lipid metabolism, according to GCRC investigators at the University of Cincinnati, the University of Southern California, and Northwestern University in Chicago, are exercise and the administration of intraluminal gastrointestinal cholesterol-binding resins and a variety of other drugs. Long-term benefits, however, have not yet been established.

Exercise may also be of therapeutic value in combating other complications of the disease. Rockefeller University GCRC investigators have reported that a program of exercise, diet, home monitoring of glucose, and self-adjustment of frequent injections of regular insulin provided markedly improved blood glucose control, similar to that achieved with an insulin pump. The program produced significant reductions in capillary basement membrane thickness and blood pressure in a group of 10 type I diabetics. The most important unresolved question in the management of diabetes, however, is the relationship, if any, between the development of the microvascular and neuropathic complications of the disease and the regulation of blood glucose, according to the report.

Research indicates that good control of blood glucose may limit complications. At the Washington University GCRC in St. Louis, researchers have found that abnormal leakage of the dye fluorescein into the vitreous of the eye after injection of the dye into the circulatory system may enable the earliest possible detection of change in diabetic retinopathy. This leakage, which probably represents breakdown of the blood-retinal barrier, was noted in diabetics who had normal fluorescein angiography. Investigators at Washington University and the State University of New York in Brooklyn have discovered that rigid control of blood glucose by using an insulin pump for 6 months significantly reduced leakage of fluorescein into the vitreous in a group of type I diabetics. This suggests that the defect is reversible, and it supports the concept that microvascular changes can be prevented or delayed by meticulous control of blood glucose.

Good control of blood glucose may be important in preventing platelet abnormalities or changes in coagulation that may lead to the genesis of such complications. Rockefeller University GCRC researchers have recently demonstrated that type II diabetics with hyperglycemia have significant reduction of fibrinogen survival. This abnormality was reversed with appropriate control of blood glucose. The reduction of fibrinogen survival was not corrected by aspirin but by heparin, suggesting a defect secondary to thrombin or one of its antagonists. The absence of diabetic microvascular complications in a group of sexually developed dwarfs with a high incidence of glucose intolerance and hyperglycemia implies that growth hormone may play an important role in the start of these complications.

Diabetic nephropathy continues to be a major cause of morbidity and mortality, according to the report. One treatment gaining favor is long-term dialysis. Initially, patients with diabetic nephropathy were not accepted into long-term dialysis programs or considered appropriate candidates for transplants. A study conducted at the Peter Bent Brigham and Women's Hospital GCRC demonstrated an increase in cardiovascular disease among hemodialysis patients and recommended screening for increased renin and nephrectomy. Researchers at the State University of New York GCRC in Brooklyn have found that the daily combination of 35 grams of oxidized starch and 35 grams of activated charcoal significantly decreases the serum triglyceride and cholesterol concentrations in patients undergoing long-term hemodialysis.

The introduction of continuous ambulatory peritoneal dialysis has contributed significantly to the treatment of patients with end-stage renal disease. A team at the University of Missouri GCRC has documented the efficacy of this procedure. Northwestern University investigators found maintenance hemodialysis comparable to peritoneal dialysis. They also discovered that morbidity was the same for patients who had long-term hemodialysis and for those who had renal transplantation from a living relative. Morbidity was higher when the kidney from an unrelated cadaver was transplanted.

Chronic kidney disease can lead to renal osteodystrophy, a bone disease with problems that have been extensively investigated at the GCRC of Washington University in St. Louis. Metabolism of vitamin D was found to be abnormal in patients with chronic failure, even though absorption was unimpaired. This abnormality was corrected by renal transplantation but not by hemodialysis. It was also shown that 25-OH cholecalciferol can increase calcium ion absorption, even in the absence of renal tissue, in which 25-OH cholecalciferol is converted to 1,25-dihydroxycholecalciferol.

The role of secondary hyperparathyroidism in the genesis of renal osteodystrophy has also been investigated. Of 195 patients with chronic uremia, only 10 percent had significant symptoms, according to tests conducted in the Peter Bent Brigham and Women's Hospital GCRC. Diagnosis by X-ray examination markedly failed to detect either osteomalacia or osteitis fibrosa cystica, abnormalities present in the disorder. Hemodialysis did not cause improvement, but renal transplantation did, although some patients who did not receive transplants were helped by parathyroidectomy. Calcium treatment of 2 weeks or longer suppressed parathyroid hormone secretion somewhat but did not increase tubular reabsorption of phosphate in patients with chronic renal failure. Removing 80 percent of the parathyroid tissue aided suppression in patients who were previously resistant to suppression. These studies indicated that the parathyroid may not be autonomous, according to researchers at the Washington University GCRC.

Since long-term complications of diabetes may be delayed and perhaps prevented by very tight control of blood glucose, methods for long-term measurement control are important. Quantification of hemoglobin A_{1c} is one such method.

Research at Peter Bent Brigham and Women's Hospital and Rockefeller University GCRC's has revealed that hemoglobin A_{1c} is a minor hemoglobin component, produced slowly and continuously in the circulating red blood cell by the nonenzymatic addition of glucose to the N-terminal amino group of the beta chain. Hemoglobin A_{1c} represents about 4 percent of the total red blood cell hemoglobin in normal individuals. In diabetics the concentration is increased in direct proportion to the degree of hyperglycemia, a finding uncovered and confirmed by investigators at several institutions including Stanford University and Washington University. When a patient with poorly controlled diabetes is brought into tight control, hemoglobin A_{1c} returns to normal levels 4 to 5 weeks after control is achieved, according to studies at the Rockefeller University, Cornell University, and Beth Israel Hospital GCRC's. Researchers at Washington University discovered that levels of A_{1c} are higher when the diabetes is hard to control than when it is easy to control and that A_{1c} levels correlate inversely with C-peptide levels, another diagnostic factor.

Investigators at the University of Chicago GCRC have found that C-peptide, the segment that connects the A and B chains of insulin in the proinsulin molecule, is excised when proinsulin is converted to insulin. C-peptide is stored in the beta cell and secreted in equimolar amounts with insulin. The measurement of C-peptide in plasma and urine has provided a unique tool for evaluating residual beta cell activity because, unlike insulin, it is not cleared appreciably by the liver. Measurement of C-peptide during insulin hypoglycemia in patients suspected of having an insulin-producing pancreatic tumor has become one of the most sensitive methods for establishing the diagnosis of an insulinoma as a cause of hypoglycemia, according to investigators at the Mayo Foundation GCRC.

Control of A_{1c} levels is important during pregnancy when high levels correlate with high-birth-weight newborns, investigators at the Stanford GCRC discovered. Rockefeller researchers have found that normalization of A_{1c} levels in the first trimester leads to normalization

Investigators at the Stanford University GCRC determine the amount of high-density lipoproteins in patient samples: ***defects in lipid metabolism may put the diabetic at high risk for coronary heart disease.***

Photo courtesy of Stanford University Medical Center of the deranged gestational hormone pattern and the delivery of normal-sized, healthy infants.

Women whose diabetes is poorly controlled have difficulty conceiving and, once pregnant, they frequently abort or deliver nonviable, postmature neonates with an unusually high incidence of congenital malformations, research at the University of Pennsylvania GCRC has confirmed. To prevent malformations, tight maternal control of blood glucose is recommended. Studies, some of which were conducted at the University of Alabama at Birmingham GCRC, have shown that fetal glucose levels respond directly to changes in maternal levels. Tests at the University of Cincinnati GCRC and at other institutions have indicated that increased transfer of glucose and other metabolites from mother to fetus is the cause of congenital malformations and perinatal mortality of infants whose mothers have poorly controlled diabetes.

Malformations occur in the central nervous, cardiovascular, and skeletal systems, according to studies by investigators at the Children's Hospital of Philadelphia GCRC. Many malformations take place early, often before the seventh gestational week. Researchers at the Peter Bent Brigham and Women's Hospital GCRC have also found pancreatic hyperfunction in the presence of maternal hyperglycemia.

At the Northwestern University GCRC, studies of pathological processes in pregnant diabetics revealed that the normal human placenta is capable of hydrolyzing the peptide bonds of insulin and other peptide hormones. The researchers have also shown that maternal insulin does not cross the placenta and does not directly affect the fetus. Microscopic inspection of placenta disclosed immature villi with mild villous edema and an increased number of active villous cytotrophoblastic cells, among other abnormalities. Those last changes were present in pregnant diabetics who had only minimally abnormal metabolism.

Researchers in GCRC's at the University of North Carolina, Northwestern University, the Medical College of Wisconsin, and Washington University have studied hormonal and other changes in pregnant diabetics. Specific insulin-binding sites in human placental tissues were identified, and the ability of the human placenta to secrete hormones that caused insulin resistance was demonstrated. It was found that infants of diabetic mothers were often abnormally large, even when the mothers' diabetes was subclinical, because these women had a defective plasma insulin response. The defect was uncovered when these mothers were challenged by a glucocorticoid similar to the steroids present during pregnancy. It was discovered that pregnant diabetics were unable to increase insulin production to offset the effects of placental hormones, establishing the need for increasing amounts of exogenous insulin as pregnancy progressed.

Better control of blood glucose is the goal of many diabetes researchers. Elucidation of the insulin biosynthesis pathway, at the University of Chicago GCRC, has led to extraordinary advances in unraveling the complex mammalian protein synthesis mechanism and has culminated in the synthesis of human insulin by bacteria, using recombinant DNA techniques and cloned insulin genes. This could result in more effective and less expensive treatment.

Although immunologic rejection of transplanted pancreatic islets appears to proceed rapidly, a study completed at the University of Minnesota GCRC has shown that adult human pancreatic fragments can be maintained in tissue culture for several days with a preferential loss of exocrine cells. Dispersed pancreatic islet tissue—partially purified islets—was also autotransplanted in eight patients with chronic pancreatitis and incapacitating, intractable pain. The patients had undergone total or near-total pancreatotomy. Four of the eight did not need insulin after the autotransplantation. In the same study, seven diabetic renal allograft recipients received allografts of dispersed pancreatic islet tissue, followed by transient evidence of islet function. Reports from the Albert Einstein College of Medicine and University of Minnesota GCRC's indicated that a number of islet tissue transplants resulted in a markedly reduced need for exogenous insulin in some patients.

Another University of Minnesota trial has involved the transplantation of segmental pancreatic allografts with unligated ducts from cadavers to 10 diabetic patients who had received renal allografts because of end-stage diabetic nephropathy. Four patients have not required exogenous insulin, one for as long as 16 months. It is not yet known whether arrest or reversal of related vascular changes has occurred. Normal glucose metabolism was apparently maintained for more than 2 years in another experiment involving two recipients of primarily vascularized allogeneic pancreatic segments who had drainage of the pancreatic duct through the ureter. Immediately vascularized segmental pancreatic allograft transplants, according to investigators at the University of Minnesota GCRC, are now thought to be more efficient than islet transplants, although the main risk of any pancreas transplantation is related to the immunosuppressive therapy necessary to stop rejection.

Perhaps the best hope for diabetics is the promising research for highly effective control of blood glucose through various insulin delivery systems. In the early 1970's a number of investigators began to explore the possibility of improving blood glucose levels by continuous infusion of insulin, with augmentation prior to meals. In studies at the Mayo Foundation GCRC with insulin-dependent diabetic patients, continuous open-loop intravenous insulin infusion with a supplemental insulin bolus during meals produced improved glucose levels, compared to conventional injection therapy. A Yale University GCRC research team has controlled plasma glucose by open-loop subcutaneous insulin administration in seven ambulatory juvenile insulin-dependent patients. The University of New Mexico GCRC investigators have demonstrated some advantages of open-loop intraperitoneal insulin delivery including absorption of most o the intraperitoneally administered insulin and avoidance of peripheral hyperinsulinemia. A number of GCRC's have reported benefits of open-loop delivery, including significant reductions in serum cholesterol, triglycerides, and fatty acids in the blood.

Probably the therapy most involved with high technology is the artificial beta cell, which consists of a glucose sensor component a variable insulin infusion system, and a computer containing an appropriate algorithm, permitting sensing of glucose concentration to be translated instantaneously into adequate insulin or glucose infusion. A number of extracorporeal artificial beta cells have been fabricated in different laboratories. Investigators at Washington University have developed an enzymatic glucose sensor. Applications of the artificial cel have varied, but the ultimate goal is to develop a completely implantable cell.

The complete report on research into diabetes mellitus is available from the General Clinica Research Centers Program of the NIH Division of Research Resources. For a copy of the report write to:

Dr. William DeCesare
Director, GCRC Program
Room 5B-51
Building 31
National Institutes of Health
Bethesda, Maryland 20205.

Information Exchange

Research Highlights

Marked differences in susceptibility to *Encephalitozoon cuniculi* among selected inbred mouse strains were found in a study by researchers at the University of Texas Health Science Center at Dallas and the University of Illinois College of Veterinary Medicine in Urbana.

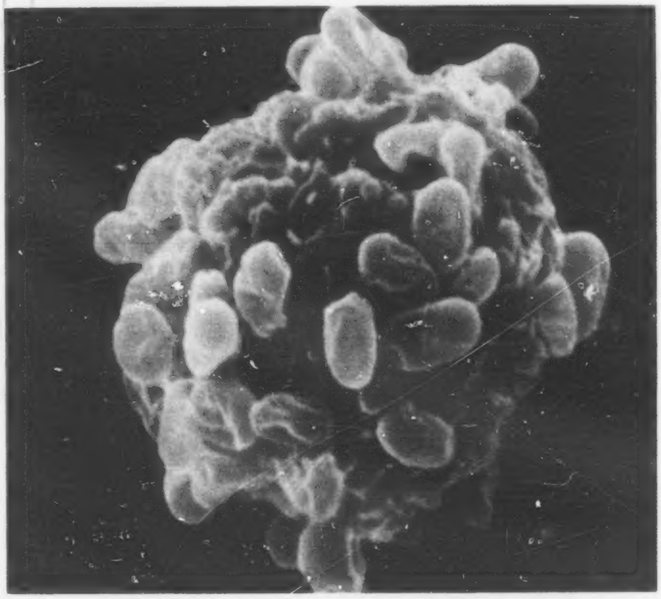
E. cuniculi is an obligate, intracellular protozoan parasite that infects a wide variety of mammals. Infections are usually asymptomatic and chronic, however, and fatalities are rare. "The infection presents a unique problem to scientists using laboratory animals," says Dr. Jerry Y. Niederkorn at the University of Texas, "and raises the question whether spontaneously infected experimental animals that appear healthy are valid experimental subjects." Animals usually become infected after ingesting spores formed by the parasite. These spores, which had been shed in the urine of other animals, form complete and active protozoa in the host cell, producing infection. Since latent infections can be activated by hydrocortisone and various other immunosuppressants, it has been suggested that the immune system may be important in controlling the infection.

In the study conducted by the principal investigator Dr. Niederkorn and Dr. John A. Shadduck and Ms. Elizabeth C. Schmidt of the University of Illinois, 11 inbred strains of mice were used to explore the immune system's role in resistance to the parasite. Susceptibility was determined by calculating the number of parasites and infected peritoneal cells (PC's) collected by peritoneal lavage 2 weeks after intraperitoneal infection with the parasite.

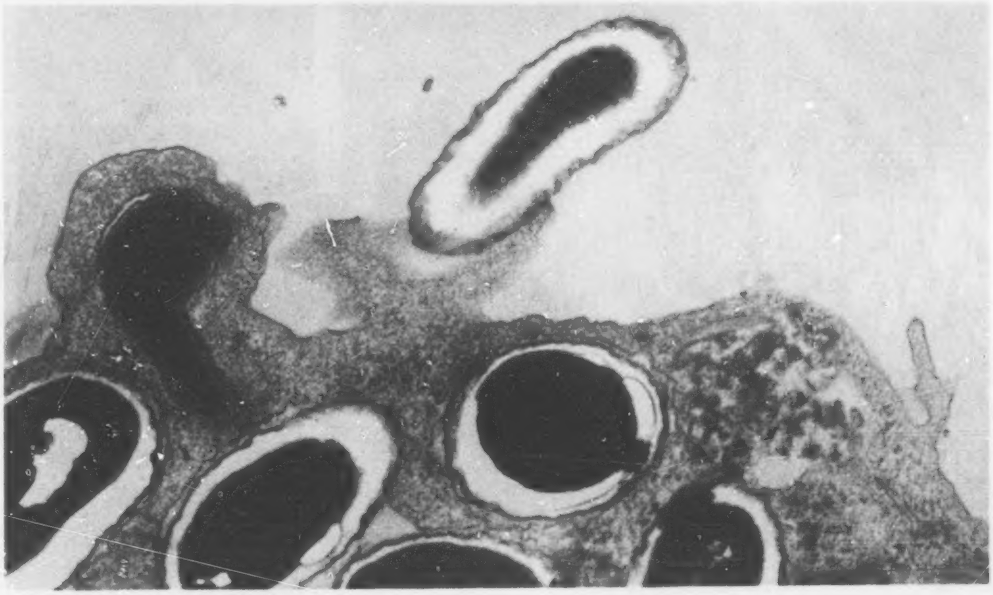
Strains that had more than 1 million parasites per mouse and more than 15 percent infected PC's were classified as susceptible. Strains that had fewer than 1 million parasites per mouse and fewer than 5 percent infected PC's were categorized as resistant. Strains that did not fit into either category were classified as intermediately susceptible.

According to these criteria, BALB/c, A/J, SJL, and C57BL/6-x-BALB/cF$_1$ strains were found to be resistant; 129/J, DBA/1, and C57BL/6 strains were classified as susceptible; and AKR, DBA/2, and C57BL/10 strains were intermediate in resistance. In contrast to the other strains, infections in hypothymic nude mice were uniformly fatal, suggesting that resistance is dependent on T lymphocytes.

Antibody response to injected sheep red blood cells and multiplication of spleen cells in response to T and B lymphocyte mitogens were used by the researchers to probe deeper into the effect of the infection on the immune system. Mitogens are substances that stimulate lymphocytes to proliferate. Typical resistant (BALB/c) and susceptible (C57BL/6) strains were studied over a 7-week period of infection.



Spores of *E. cuniculi* bound to the membrane of a macrophage: *trapped for engulfment by the macrophage. Most infections occur from the spore stage of the parasite.*



Macrophage engulfing *E. cuniculi* spores (black objects): *the body's attempt to sequester and destroy the infectious agents.*

Photos courtesy of Dr Jerry Y Niederkorn

In the resistant BALB/c strain, antibody formation was approximately equal to that of the uninfected controls, and spleen cell responses to concanavalin A, phytohemagglutinin, and lipopolysaccharide were greater in infected than in control animals. In the susceptible C57BL/6 strain, however, antibody formation was significantly depressed throughout the 8 weeks of infection, and spleen cell responses to the three mitogens declined to practically nil by the end of the seventh week. Genetic analysis of the responses indicated that susceptibility and resistance in the strains were not controlled by loci within the mouse major histocompatibility (H-2) complex.

The researchers conclude that resistance to *Encephalitozoon cuniculi* is dependent on an intact immune system—in particular, on a functional T lymphocyte component.

Additional reading:

1. Niederkorn, J. Y., Shadduck, J. A., and Schmidt, E. C., Susceptibility of selected inbred strains of mice to *Encephalitozoon cuniculi*. *Journal of Infectious Diseases* 144:249-253, 1981.
2. Niederkorn, J. Y., and Shadduck, J. A., Role of antibody and complement in control of *Encephalitozoon cuniculi* infections by rabbit macrophages. *Infection and Immunity* 27:995-1002, 1980.

Research into susceptibility of inbred strains of mice to Encephalitozoon cuniculi *was supported by the Animal Resources Program of the NIH Division of Research Resources and the Goddard Foundation.*

Research Service

Flow Cytometry Resource

Technology for rapidly measuring chemical and physical properties of cells is now available at the National Flow Cytometry and Sorting Research Resource of the Los Alamos National Laboratory in New Mexico.

The resource, supported by the Biotechnology Resources Program of the NIH Division of Research Resources, provides sophisticated instrumentation useful for experiments in cell biology, immunology, molecular biology, and radiobiology. It will soon provide a core research program; host visiting collaborative researchers; sponsor flow cytometry training courses, workshops, and seminars; and support postdoctoral training positions.

The instruments, developed by scientists at the Los Alamos National Laboratory, automate the tedious and time-consuming task of studying individual cells in a large population. Traditionally, scientists separate cells in bulk, using electrophoresis and centrifugation, but separation by these methods is often limited. In the 1960's a cell sorter that isolated cells according to differences in volume was developed, but it was not until recently that subtle differences in cells could be measured accurately with advances in flow cytometry instrumentation pioneered at Los Alamos.

Abnormal cells can be identified and removed while passing through resource instruments at a rate of up to 5,000 cells per second. Abnormal cellular components such as chromosomes can be removed from a stream of components passing through the instruments at a rate of up to 1,000 per second. In contrast, a scientist with a microscope can examine only 40 to 50 chromosomes per minute.

The process of studying cells with a flow cytometer involves marking individual cells with fluorescent stains or tags specific for a cellular property. The intensity of light scattered by a cell can be related directly to both cell size and morphology. Cell-sorting instruments can differentiate cells on the basis of distinctive fluorescent stains, cell volume, light scattering, or any combination of these parameters.

After the cells are tagged, they are suspended in a fluid that carries them through the instrument. The size and speed of the fluid stream allow only one cell at a time to pass through the sensing region of the flow chamber. Cells leave the inlet tube and are aligned and guided through the first opening, where the volume of a single cell can be measured. An electrical signal proportional to cell volume is generated as the cell passes through the opening. The signal is translated and stored by a minicomputer for

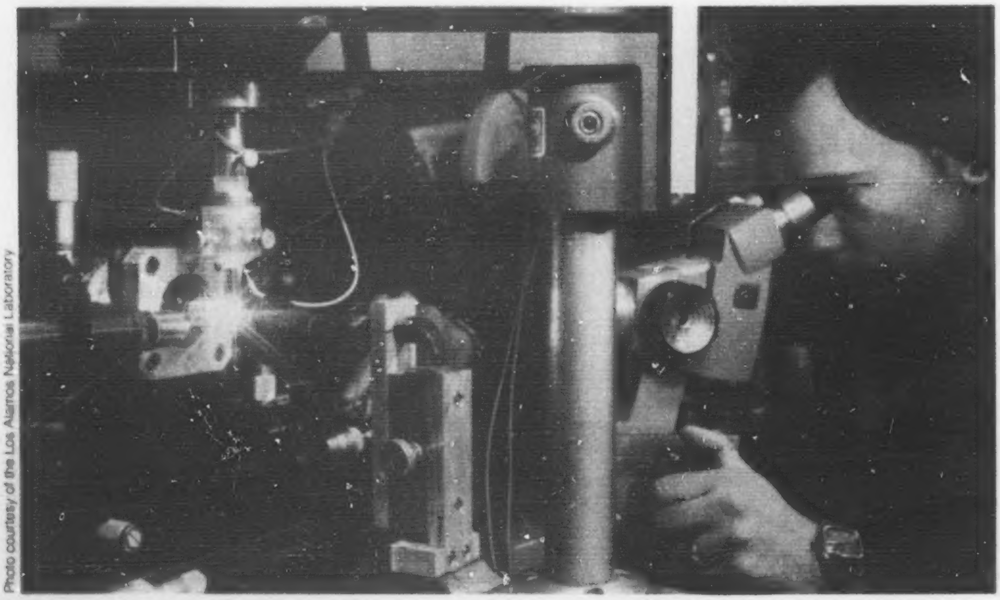


Photo courtesy of the Los Alamos National Laboratory

Scientist examines cells as they pass through a flow cytometer: *identifying and removing abnormal cells from a stream of up to 5,000 cells per second.*

later analysis. The fluid then enters another sensing region of the flow chamber, where it is exposed to a laser beam. The intensity of laser light scattered by the cell indicates cell size and structure.

Cells stained with fluorescent dyes, which bind to specific parts of the cells such as the DNA or protein, can also be measured. Light at the proper wavelength excites the dye molecules and causes specific parts of the cell to emit visible light. The intensities of these optical signals are recorded and analyzed.

When the computer receives data indicating that the cell may be abnormal, the computer initiates a sequence that applies an electrical charge to a series of droplets leaving the flow chamber. These droplets, one of which contains the selected cell, are deflected by charged plates so that they fall into a collection vessel. Those droplets containing cells that do not give the desired signal fall into a separate collection vessel. The sorted cells can be tested further.

Instruments at the Los Alamos resource were constructed by researchers there; each instrument has specialized capabilities. One instrument can use three lasers to illuminate a single cell labeled with three different fluorescent tags for different cellular constituents; another can be used to perform fast kinetic and polarization measurements.

Flow cytometry at the resource is being used to analyze normal and abnormal chromosomes, such as dicentric chromosomes, which were altered by exposure to radiation or toxic agents. Core researchers are also using flow cytometry to evaluate damage to the immune system caused by exposure to physical and chemical agents associated with developing energy technologies. In one study, researchers are evaluating changes in the natural defense mechanisms of the lungs. Scientists at the Los Alamos Scientific Laboratory also are studying the role that macrophages play in tumor cell recognition.

According to Dr. L. Scott Cram, director of the resource, the use of flow cytometry will be particularly useful in studying cancer cells. Because these cells are believed to have altered genes, scientists can use flow systems to study changes in DNA content during cell life cycle. Each phase of a normal cell's life cycle is associated with changes in DNA content. Malignant cells sometimes contain elevated amounts of DNA and tend to be intermediate in size. Studying the ability of certain antigens or antibodies to bind on cell surfaces may also provide information about tumor cells.

Scientists interested in using flow cytometry technology may submit project descriptions to the resource for approval.

For further information, contact:

Dr. L. Scott Cram
Director
National Flow Cytometry Resource
Experimental Pathology Group, MS-888
Los Alamos National Laboratory
Los Alamos, New Mexico 87545
Telephone (505) 667-2785.

Note on PROPHET

In the June 1982 issue of the *Research Resources Reporter* the article entitled "PROPHET: A Network for Analyzing and Sharing Research Data" incorrectly lists the Oregon Regional Primate Research Center in Beaverton as being linked to the PROPHET network. The Oregon State University School of Pharmacy in Corvallis is linked to PROPHET as are the Regional Primate Research Center at the University of Washington in Seattle and about 35 other colleges, laboratories, and research institutions.

Meetings

Clinical Applications Of Biomaterials

An NIH Consensus Development Conference on the Clinical Applications of Biomaterials will be held November 1 to 3, 1982, in Bethesda, Maryland. The conference will provide an open forum for biomedical investigators, practicing physicians, manufacturers, and consumers to discuss and evaluate the safety and effectiveness of drugs, devices, and procedures currently in clinical use. Conference participants also will discuss whether biomaterials used today fulfill the needs of the clinician, and they will assess the methods for introducing biomaterials into clinical care. The conference will consist of a series of presentations by experts and an open discussion among panel members and the audience.

For more information, contact:

John W. Boretos
Building 13, Room 3W13
National Institutes of Health
Bethesda, Maryland 20205
Telephone (301) 496-5771.

October 14-15, 1982

Title: *13th Annual Neural Prosthesis Workshop*
Location: *Bethesda, Maryland*
Contact: *Dr. Terry Hambrecht*
Neural Prosthesis Program
Federal Building, Room 916
NINCDS
National Institutes of Health
7550 Wisconsin Avenue
Bethesda, Maryland 20205
Telephone (301) 496-5745

October 16-20, 1982

Title: *19th National Reticuloendothelial Society Meeting*
Location: *St. Louis, Missouri*
Contact: *Dr. Sherwood M. Reichard*
Reticuloendothelial Society
Medical College of Georgia
Augusta, Georgia 30912
Telephone (404) 828-2601

October 18-19, 1982

Title: *Chromosomes and Cancer: From Molecules to Man*
Location: *Chicago, Illinois*
Contact: *Dr. Janet D. Rowley*
The University of Chicago
Cancer Research Center
Box 444
950 East 59th Street
Chicago, Illinois 60637
Telephone (312) 947-6386

October 18-21, 1982

Title: *33rd International Instrumentation and Automation Conference and Exhibit*
Location: *Philadelphia, Pennsylvania*
Contact: *Mr. C. T. Glazer*
Meeting Coordinator
Instrument Society of America
67 Alexander Drive
Research Triangle Park, North Carolina 27709
Telephone (919) 549-8411

October 23-27, 1982

Title: *3rd Congress of the International Society for the Study of Hypertension in Pregnancy*
Location: *Dallas, Texas*
Contact: *Dr. Marshall D. Lindheimer*
Professor of Obstetrics and Gynecology
University of Chicago
950 East 59th Street
Chicago, Illinois 60637
Telephone (312) 947-1728

October 30-November 5, 1982

Title: *24th International Congress of Ophthalmology*
Location: *San Francisco, California*
Contact: *Meeting Coordinator*
P.O. Box 3030
San Francisco, California 94119
Telephone (415) 921-4700

November 3, 1982

Title: *Anorexia Nervosa: Causes and Cures*
Location: *New Hyde Park, New York*
Contact: *Ann J. Boehme*
Office of Continuing Education
Long Island Jewish-Hillside Medical Center
New Hyde Park, New York 11042
Telephone (212) 470-2114

November 7-10, 1982

Title: *3rd Biennial Clinical Conference of the National Perinatal Association*
Location: *Dallas, Texas*
Contact: *Kathy Hunter*
1311-A Dolly Madison Boulevard
McLean, Virginia 22101
Telephone (703) 556-9222

November 10-12, 1982

Title: *5th Chemotherapy Foundation Symposium on Innovative Cancer Chemotherapy Tomorrow*
Location: *New York, New York*
Contact: *Director*
Page and William Black Postgraduate School of Medicine
Mount Sinai School of Medicine
Annenberg 5-206
One Gustave Levy Place
New York, New York 10029
Telephone (212) 650-6737

November 11-15, 1982

Title: *16th Annual Congress of the American Association for Clinical Immunology*
Location: *Anaheim, California*
Contact: *H. Silber*
P.O. Box 912
Omaha, Nebraska 68101
Telephone (402) 551-0801

November 14-18, 1982

Title: *33rd National Meeting of the American Pharmaceutical Association Academy of Pharmaceutical Sciences*
Location: *San Diego, California*
Contact: *Office of the Executive Secretary*
Academy of Pharmaceutical Sciences
2215 Constitution Avenue, N.W.
Washington, D.C. 20037
Telephone (202) 628-4410

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Research Resources Reporter

November 1982, Volume VI, No. 11

U.S. DEPARTMENT OF HEALTH
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Serving Animal Resources,
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General Clinical Research Centers,
Minority Biomedical Research Support,
and Biomedical Research Support

This Issue:

Peritoneal Drug Delivery
Adult Hyperactivity
SLE and Autoimmunity
Hormone Influences Cancer

by
Greg Freiherr

Medical Expertise Applied to Dental Research

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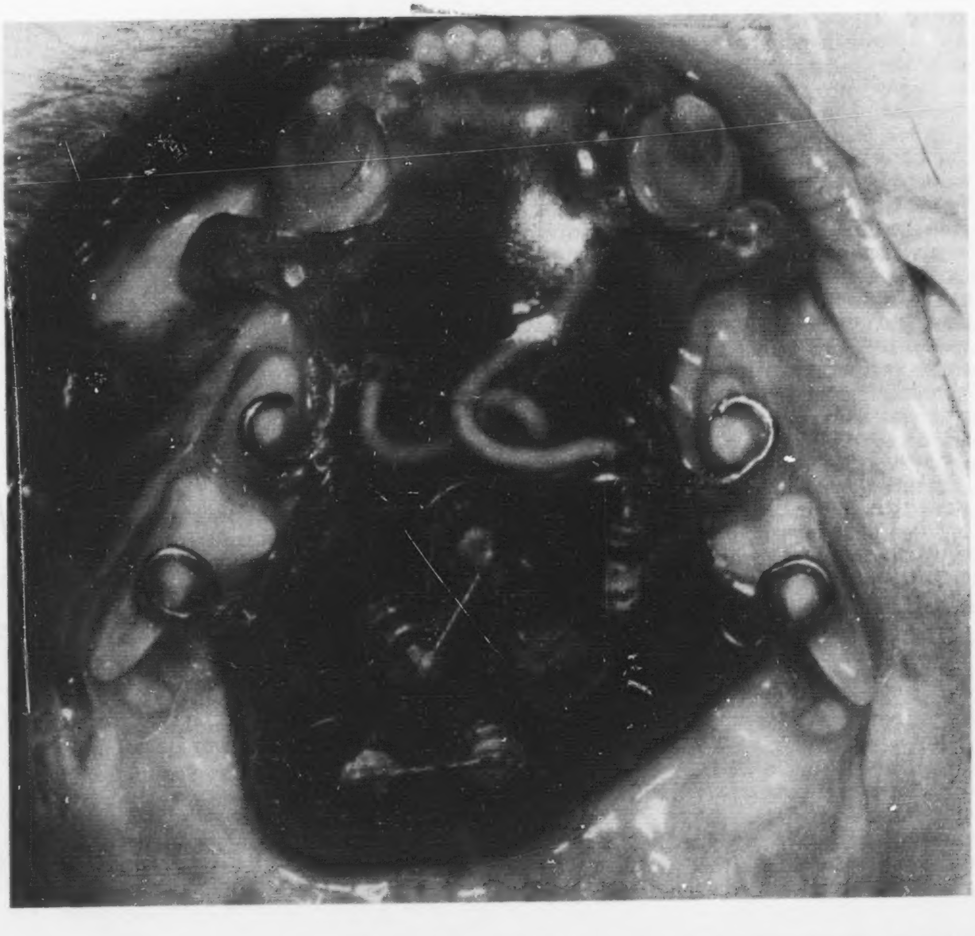
Pathologists examine the behavior of bacteria to determine which ones damage teeth, gums, and the heart. A physicist, a biochemist, and an epidemiologist look for signs of toxicity in the dentist exposed to mercury. Engineers develop sensors to monitor dental health and metabolic disease. Electrical techniques developed by orthopedists to speed the healing of fractures are modified to straighten teeth more efficiently.

"These and other efforts going on at the Dental Clinical Research Center demonstrate the interdependence of dentistry and medicine," says Dr Irwin Ship, professor of oral medicine at the University of Pennsylvania in Philadelphia.

As director of the university's W. D. Miller Dental Clinical Research Center (DCRC), Dr. Ship and the center's principal investigator, Dr. D. Walter Cohen, promote studies in which expertise and knowledge gained in traditionally medical disciplines are used to



Dental appliance that selectively administers electricity to teeth: ***first step toward reducing the time orthodontic braces must be worn?***

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DCRC *continued*

improve dental health care. "What physicians know about human physiology and pathology gives us a better understanding of diseases that primarily affect oral tissue," says Dr. Cohen, dean of the University of Pennsylvania School of Dental Medicine.

Physicians agree that poor management of metabolic diseases may lead to serious complications. Fluctuating serum glucose levels in diabetics may cause eye or kidney problems. Diabetes may also cause the inflammation and destruction of tissues that surround and support the teeth, a condition called periodontitis. Dr. Robert Friedman, assistant professor of oral medicine at the University of Pennsylvania, says that diabetics generally have an unusual degree of periodontal disease, suggesting that better management of diabetes could reduce the incidence and severity of oral disease in these patients.

Studies done at the DCRC by Dr. Friedman show that diabetics have abnormally high levels of fluid glucose in the gingival crevice, the space between the teeth and the gums. Recent studies at the DCRC revealed that not only the glucose level in the crevice but also the volume of gingival crevice fluid (GCF) varies according to the serum level of glucose, a finding that may allow physicians to easily gauge the effectiveness of management therapy for diabetes. Currently a blood test is the only means available to physicians for determining serum glucose levels. But with a microcapillary tube made of silicon, Dr. Friedman can record the volume and composition of GCF and painlessly, simply, and accurately calculate the level of GCF glucose.

The next logical step is to monitor GCF glucose automatically. Prototypes of electronic sensors that can be placed unobtrusively in the mouth are already being developed by the DCRC in conjunction with the university's dental bioengineering center and chemical electronics center. Sensors now being designed will specifically monitor pH, calcium, phosphate, fluoride, or glucose. Dr. Jay N. Zemel, director of the chemical electronics center, hopes that a second generation of devices can be built to monitor several chemicals or chemical factors simultaneously. These sensors would be used in a variety of experiments that would not constrain patients to a laboratory environment and would make it possible to examine the interaction of plaque, caries, and food in natural or realistic experiments. "What is known about the formation of caries is based on laboratory data and a lot of theory," says Dr. Samuel L. Yankell, a University of Pennsylvania biochemist responsible for testing and evaluating the sensors. "Almost nothing is known about what actually happens in plaque."

Photo by Greg Freiherr

Dr. D. Walter Cohen, dean of the University of Pennsylvania School of Dental Medicine and principal investigator of the DCRC: *"What physicians know about human physiology and pathology gives us a better understanding of diseases that primarily affect oral tissue."*

At present Dr. Zemel and his team are attempting to prove the feasibility of oral chemical sensing with the development of sensors for the calcium level and the pH of plaque. The presence of food, particularly sugar, affects the growth of bacteria and the pH of plaque. As the plaque pH becomes more acid, calcium in the tooth begins to dissolve, and calcium ions appear in plaque and saliva. Correlating plaque pH and calcium level with bacterial activity would provide a good measure of the patient's dental health and might eventually be used to improve diagnosis, therapy, and management.

But such correlates will rely on rapid and reliable measurements. Available methods are inadequate. They involve scraping plaque from teeth, placing bulky electrodes on or into plaques with wires running out of the mouth, or the use of existing pH telemetry sensors, which require space from missing teeth and are, therefore, limited mainly to use with elderly patients.

The pH sensor being developed at the DCRC should overcome these problems. It is designed to be built into removable dental appliances or orthodontic brackets. Initially, wires will link the sensor to recording instruments outside the mouth, but these will ultimately be replaced by a miniature, battery-powered telemetry unit that will transmit data to

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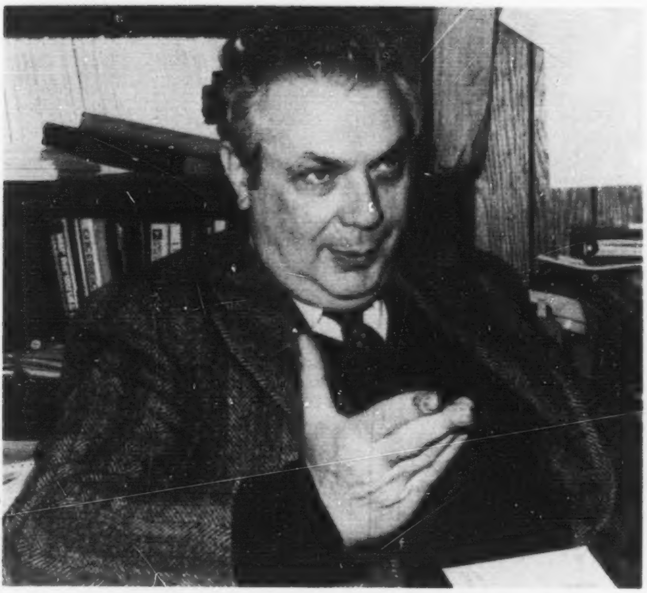

Photo by Greg Freiherr

Dr. Jay N. Zemel, director of the University of Pennsylvania chemical electronics center: *developing sensors to correlate biochemical activity with oral disease.*

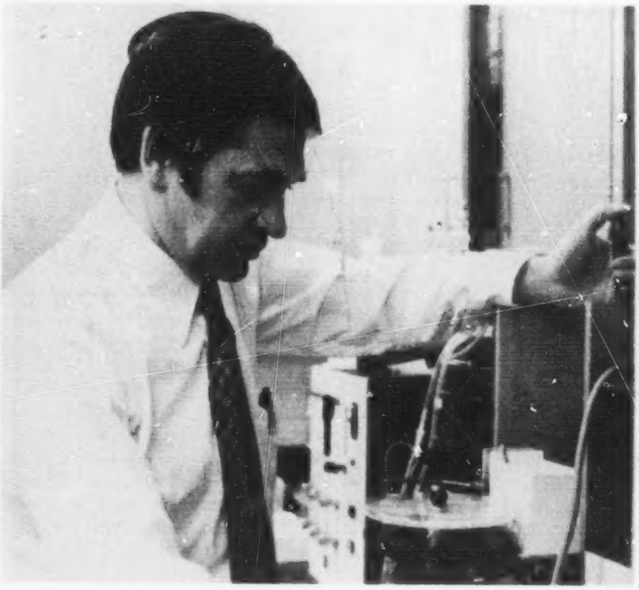

Photo by Dr. John Hayden, University of Pennsylvania Health Affairs

Dr. Irving Shapiro: *assessing the levels of mercury in dentists and their assistants.*

receivers in the laboratory. Clinical testing of the sensors will be done at the DCRC, where collaboration was initiated between dental researchers and engineers in the University of Pennsylvania School of Engineering and Applied Sciences. "Although microsensors are research tools at this time, they will certainly contribute to preventive dentistry by allowing dentists to monitor chemical changes that precede the destruction of gums and teeth," Dr. Zemel says.

Correlation between these indicators and the prevalence of dental disease, particularly cavities, could provide standards for such products as food additives, chewing gum, candy, cookies, and cereals. These and other food products serve as the medium on which oral bacteria feed. Brushing, flossing, and regular professional cleanings are needed to keep bacterial plaque at a manageable level. Checkups are essential. "Even advanced stages of periodontal disease are often painless, and development of the disease is slow," Dr. Ship says, "so that it goes unnoticed by the patient."

Gums first become tender and swollen with occasional bleeding around the teeth, a condition called gingivitis. Next the gums change from pink to an unhealthy red. In advanced stages, the gums recede permanently and bone supporting the teeth is damaged.

"Approximately 90 percent of the adults in the United States have at least mild periodontal disease," says Dr. Max Listgarten, professor of periodontics at the University of Pennsylvania. "Most cannot avail themselves of extensive treatment because of the expense, so prevention of serious cases by quickly identifying the early stages of the disease and applying treatment is extremely important."

Dr. Listgarten, an expert in the cellular pathogenesis of periodontal disease, Dr. Norton Taichman, chairman of the pathology department of the University of Pennsylvania, and their colleagues are now trying to determine if differential bacteria counts are indicators of disease activity. "If they are, we want to use these counts to determine how frequently patients should have their teeth cleaned," Dr. Listgarten says.

The researchers are particularly interested in two types of bacteria—spirochetes and motile rods. Research done at the DCRC documents an association between elevated levels of these two types of bacteria and the presence of periodontitis, the most advanced stage of periodontal disease. At this stage, pockets of the bacteria have severely damaged the tissue and bone that support teeth, causing gums to recede permanently and teeth to loosen. Often these teeth must be extracted.

To prevent the onset of periodontitis, plaque must be scraped from the mouth regularly. Research indicates that teeth cleaning every 2 weeks controls the repopulation of bacteria. Annual cleaning does not. "For most patients, cleanings every 2 weeks are prohibitively expensive and not needed. Arbitrary recalls every 6 months may not be soon enough for some, but they may be too frequent for others," Dr. Listgarten says. "By measuring the repopulation rates of spirochetes and motile rods in each patient, recording the proportions of these microorganisms, and comparing the data to levels associated with tissue damage, it should be possible to establish optimal recall intervals."

Dentists could then concentrate on patients who need the most attention, reducing the costs of dental care for disease-resistant patients and intervening early to reduce or eliminate the cost of therapy for patients who are susceptible to disease.

Dr. Listgarten and his colleagues are now studying the

DCRC *continued*

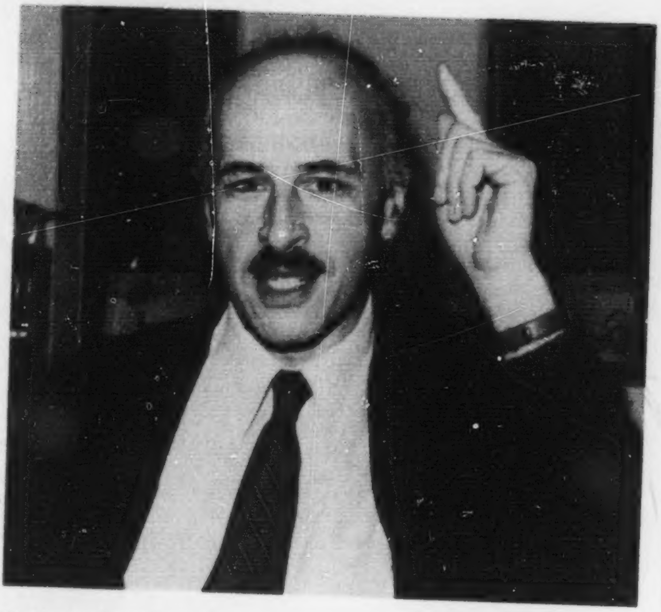

Photo by Greg Freiherr

Dr. Norton Taichman, chairman of the pathology department at the University of Pennsylvania and researcher of juvenile periodontitis: *"I believe we are on the verge of a breakthrough in being better able to diagnose, treat, and perhaps prevent this disease."*

Photo by Greg Freiherr

Technician builds dental appliances for DCRC research: *enhancing dental research with biotechnology.*

repopulation rates of bacteria in some 60 patients. Half of these patients follow individually tailored cleaning schedules. The other half, who serve as the control group, receive cleanings twice yearly, a schedule commonly recommended by practicing dentists.

The researchers are tailoring schedules according to a test that Dr. Listgarten describes as being "remarkably simple." "Just scrape bacteria from the mouth, count the first 200 cells, and come up with a proportion of spirochetes and motile rods in this field," he says. "Any well-trained hygienist can learn to do it."

As a result of this test, some DCRC patients have not had their teeth cleaned since the study began 3 years ago nor have they shown any tooth or gum damage. Others have required cleanings every 2 to 3 months.

Comparing the dental health of the experimental and control groups will determine the benefits of a customized schedule versus the arbitrary 6-month recall program. Results now favor the tailored schedule. If these preliminary findings are confirmed, the study could lead to a preventive regimen that would save time and money as well as improve oral health, Dr. Listgarten says.

But even the most rigorous preventive and therapeutic treatments sometimes cannot stop severe periodontitis from occurring, particularly the type that attacks children and young adults. Dr. Taichman and his associates suspect that immune defects allow the rampant destruction of gum and bone that is characteristic of juvenile periodontitis (JP), a disorder that often leads to the loss of teeth during adolescence. The disease occurs rarely, but when it does, little can be done to stop it. "Juvenile periodontitis causes untold emotional, physical, and economic hardship for patients and their families," Dr. Taichman says.

He theorizes that the response and activity of antibodies and white blood cells in these children is weaker than normal. When the rod-shaped bacterium *Actinobacillus actinomycetemcomitans* (AA) invades the gingival crevice of these patients, it releases a toxin that destroys most or all of the white blood cells in the area. Resultant tissue damage and the proliferation of the bacteria cause serum to leak into the crevice. The serum feeds the bacteria, and toxin production increases.

Antibody response is slow and is neutralized by the toxin, according to Dr. Taichman. White blood cells and monocytes from elsewhere in the body never reach the infected gingival crevice or do so in limited and inconsequential numbers.

Without an effective counterattack, AA and other bacteria proliferate wildly and inflict heavy tissue damage. The disease accelerates, spreads to other sites, and the cycle is repeated. If not controlled, juvenile periodontitis ultimately destroys the bone that supports the teeth. According to reported cases, patients have lost some or all teeth between ages 12 and 30.

Clinical and laboratory studies conducted at the DCRC and elsewhere support Dr. Taichman's theory of the role of AA. Although pockets of the disease harbor a complex mixture of bacteria, AA is present in commanding numbers; antibody levels in the sera and the gingival crevices of patients with JP are high; and AA rapidly induces bone loss in the tooth sockets of laboratory animals. The studies have shown that a strain of the bacterium called Y4, which is present in JP patients, produces two highly active toxins—one that promotes bone resorption and another that destroys white blood cells and monocytes.

"A majority of the children with clinically detectable JP show signs of having been infected with AA," Dr. Taichman says. "The disease is localized

Dental hygienist at the University of Pennsylvania DCRC counts bacteria in a plaque sample: *applying biomedical expertise to research in dentistry.*

to only a few teeth in patients who develop a high titer of antibodies against the Y4 strain of AA, whereas the disease involves many teeth in those who do not develop a good immune response."

Dr. Taichman and his colleagues at the DCRC hope to characterize the disease precisely with a 5-year project involving 40 subjects with JP. During the study, patients will be placed on a program of preventive cleanings and checkups similar to the program established at the DCRC for average patients. "Our JP subjects have all had surgical treatment for periodontitis and are much more susceptible to disease than are average patients," he says. "Now that we have some experience with selective cleaning schedules for average patients, we are more confident in dealing with JP subjects."

While battling the disease with dental hygiene, the researchers will evaluate tissue response to the therapy in these subjects. They will identify and measure the action of AA on gum, bone, and blood cells. Each patient will receive a complete medical and dental examination and will provide a family history to allow the researchers to test the hypothesis that an X-linked chromosomal defect impairs the immune system and makes the patient susceptible to JP.

"I believe we are on the verge of a breakthrough in being better able to diagnose, treat, and perhaps prevent this disease," Dr. Taichman says. "Our experiments on the mechanism of leukocyte interaction with AA may help to accelerate this objective and to generate data useful for investigating other diseases."

Before Dr. Taichman and his colleagues began studying juvenile periodontitis, AA was described in the medical literature as being associated with heart valve disorders. Recently the DCRC team helped physicians at a local hospital to identify this organism, which was found on the heart valves of two patients.

Dr. Taichman suggests that the bacterium enters the bloodstream after successfully attacking and destroying tissue in the mouth. "We believe that the two patients at the hospital may have had juvenile periodontitis," he says. "We couldn't confirm the diagnosis because we didn't see the children, and the physicians are not educated to recognize JP; but all the clinical signs of JP seemed to be present."

Just as the study of bacteria in the mouth may yield information useful in medicine, research into medical problems sometimes impacts on the dental community. A method called X-ray fluorescence radiography was applied by Dr. Irving Shapiro, professor of biochemistry, and Dr. Peter Bloch, associate professor of radiological physics, both at the University of Pennsylvania, to measure lead in the teeth of children suspected of being poisoned by paint chips.

"Then one day, Peter and I were discussing a newspaper article that described dentists as being high insurance risks because of their rate of suicide. The article also cited dentists as having a high divorce rate and being prone to depression," Dr. Shapiro says. "We wondered if dentists had problems caused by inhaling mercury vapors. We wondered, in fact, if dentists were another form of mad hatters—hat makers in the 1800's who went crazy because they absorbed mercury used to soften the felt in hats."

Despite the development of new composite materials for dental use, most fillings include an amalgam of silver and mercury. A busy dental office may consume 3 pounds of mercury each month, according to Dr. Shapiro. The standard practice of handling mercury in premixed capsules has reduced much of the danger, but spills and the resulting inhalation of mercury vapors still occur.

The biological effects of mercury are not known exactly, but the liquid metal has been implicated in senile dementia and amyotrophic lateral sclerosis. "So we decided to use the X-ray fluorescence technique to measure the amount of mercury in the wrists and brains of dentists," Dr. Shapiro says.

With minor modifications, the technique was ready for application to the new problem. "Dr. Shapiro and Dr. Bloch had the equipment, methods, and techniques but lacked access to the exposed patient population," says DCRC Director Dr. Ship. "In this project, the DCRC's role was to serve as a consultant in epidemiology and to recruit the participants."

The center recruited 300 dentists, aged 45 to 64, from Pennsylvania, Delaware, and New Jersey. It also collected demographic and health information. Mercury levels in each subject were assessed at the DCRC and examined in Dr. Shapiro's laboratory.

Of the dentists studied, 18 with the highest levels of mercury accumulation showed abnormal neurological reactions. These were attributed to previous medical conditions in only five of the dentists. Those with low levels of mercury showed no neurological abnormalities.

Several dentists had mild, subclinical cases of carpal tunnel syndrome, a neurological disorder causing numbness in the lower arm and hand. Individuals with the highest mercury levels showed the most psychological distress as well as visual and perceptual changes. The researchers concluded that use of mercury as a restorative material can result in long-term health risks to dentists; but they stopped short of saying that any subjects in the study were actually poisoned.

"We showed that some apparently normal dentists have neurological dysfunction due to mercury," Dr. Shapiro says. "Whether the mercury interferes with their personal or professional performance—their

DCRC *continued*

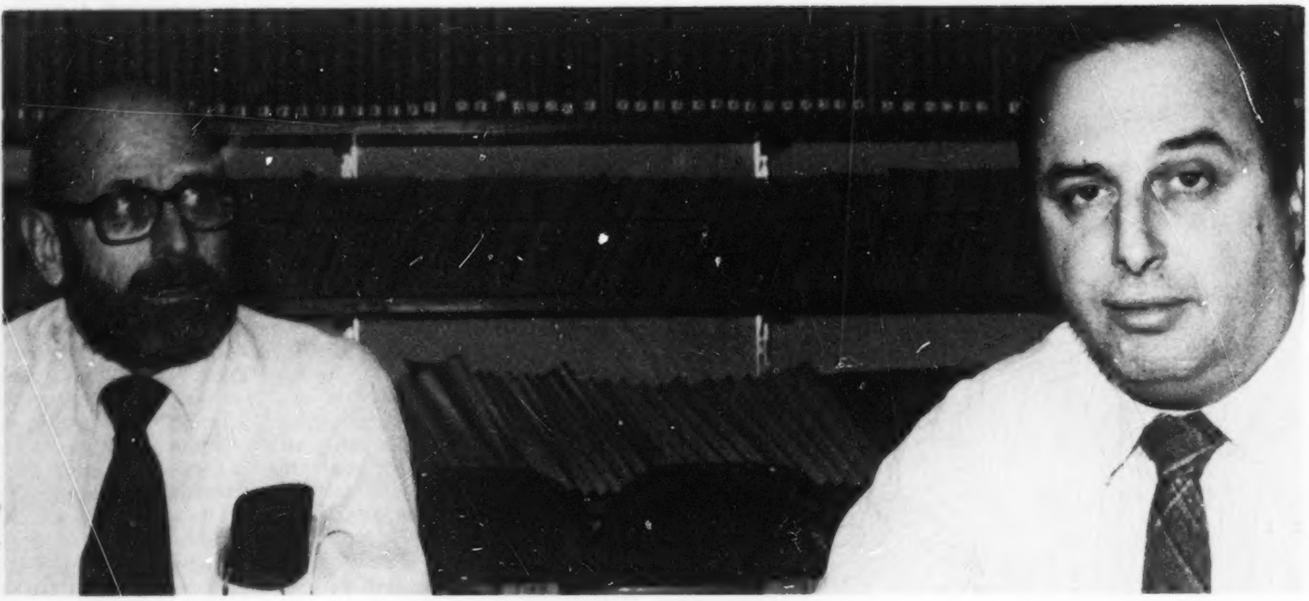

Photo by Greg Freiherr

Drs. Edward Korostoff (left) and Zeev Davidovitch of the University of Pennsylvania dental bioengineering center: *electricity to aid straightening of teeth.*

ability to repair holes in teeth and interact well with patients, friends, and relatives—is very difficult to evaluate. Our project was set up to examine a suspected relationship between mercury exposure and various aspects of neurophysiological function."

The risks of mercury exposure can be reduced by modifying the dental office, the researchers say. Tests for mercury in 54 offices revealed that the vapor level of the liquid increases sharply when linoleum floor covering is scuffed or carpeting is present in the area where restorative materials are prepared. Scuff marks and carpeting provide reservoirs in which spilled amalgam powder can reside. Controlled testing of these reservoirs by the DCRC researchers showed that mercury vapor levels increased 18 times above the ambient level when scuff marks were present and 28 times above the ambient level when floors were carpeted. Anyone exposed to mercury vapors from these reservoirs could be affected, which is why Drs. Shapiro and Bloch are now applying the X-ray fluorescence technique and a battery of neurophysiological tests in a followup study of the dental auxiliaries who prepare restorative materials.

Just as experience gained from medical studies was applied directly to the mercury study, so the use of electricity to speed the healing of fractures was applied to straightening teeth. At the University of Pennsylvania dental bioengineering center, Dr. Zeev Davidovitch, professor of orthodontics, and Dr. Edward Korostoff, professor of restorative dentistry, are collaborating in the design of a dental appliance that selectively administers electricity to teeth that are being mechanically moved. Dr. Davidovitch states that the electrical appliance is not designed to replace mechanical orthodontics. "The goal is simply to reduce the time needed for orthodontic treatment," he says.

The appliance consists of several batteries, a transistor, a resistor, a stainless steel cathode, and a gold-wire anode. Each component is embedded in acrylic. Only the tips of the electrodes are exposed. The anode and cathode are positioned on opposite sides of the tooth. Electricity flowing between the electrodes causes bone to form on one side of the tooth and to be resorbed on the other. According to the researchers, this formation and resorption of bone is mediated by changes in the normal levels of cyclic adenosine monophosphate (AMP) and cyclic guanosine monophosphate (GMP), two compounds present in periodontal ligaments and alveolar bone cells. Changes in AMP and GMP levels occur when wire and springs move teeth, the researchers say. Electrical stimulation accentuates the changes and thereby increases the rate of tooth movement, they believe.

Tests conducted for 7 to 14 days on female cats resulted in a 100 percent increase in the rate of tooth movement—an actual movement of 1 mm with the addition of electricity compared to 0.5 mm with only conventional braces. "The experiments were short-term because the cat is not exactly enthused about wearing this appliance," Dr. Korostoff says. "It is designed primarily for the human mouth and is, therefore, too large for the cat. Another problem is that the cat cannot bite properly and if the appliance is left in for very long, eating is disrupted, and poor nutrition could affect the response to electrical stimulation."

The researchers have now begun applying the technique clinically. They will install appliances in 30 subjects grouped according to age. Conventional orthodontic procedures will be modified with the placement of electrodes on one of the two canine teeth in

Photo by Greg Freiherr

Dr. Irwin Ship, director of the DCRC: *"Hopefully, collaboration between researchers in dentistry and medicine will better inform practitioners in both disciplines on how cooperation and shared knowledge can benefit their patients."*

each subject, allowing researchers to determine the rate of movement with and without electrical stimulation in the same patient. Thirty patients, similarly grouped, will serve as controls, receiving no electrical stimulation.

Starting 2 weeks after tooth extraction, electricity will be applied in the study group for 8 hours per day for 12 weeks. The entire course of treatment will continue for the usual 2 to 3 years. Tooth movement will be evaluated during and immediately following electrical stimulation and periodically thereafter.

The chance of tissue damage resulting from the application of electricity is all but discounted by the researchers. "Unlike drugs, which affect the entire body, direct electrical current has only a local effect, and we have seen no adverse local reactions in animal tissues," Dr. Davidovitch says. Careful examinations of the gums will be conducted regularly, however, to ensure that adverse effects do not occur.

Design of the appliance and methods for the study were drawn from the experience of orthopedic researchers at the University of Pennsylvania who have applied electricity extensively as an aid in the healing of fractures. "Coordinated efforts by medical and dental specialists are required," says Dr. Cohen, "if dental research is to achieve its full potential."

According to Dr. Ship, research at the center exemplifies what can be achieved when the dental and medical disciplines work together. "Hopefully," he says, "collaboration between researchers in dentistry and medicine will better inform practitioners in both disciplines on how cooperation and shared knowledge can benefit their patients."

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Research into the repopulation rates of bacteria affecting oral tissue is funded by the National Institute of Dental Research. Study of juvenile periodontitis is supported by the National Institute of Dental Research. The study of mercury toxicity in dental personnel was funded by the National Institute for Occupational Safety and Health and the American Fund for Dental Health. Application of electricity to the movement of teeth is supported by Venture Technology, Inc. The University of Pennsylvania Dental Clinical Research Center is supported by the General Clinical Research Centers Program of the NIH Division of Research Resources.

Research Focus

Peritoneal Drug Delivery for Special Diabetics

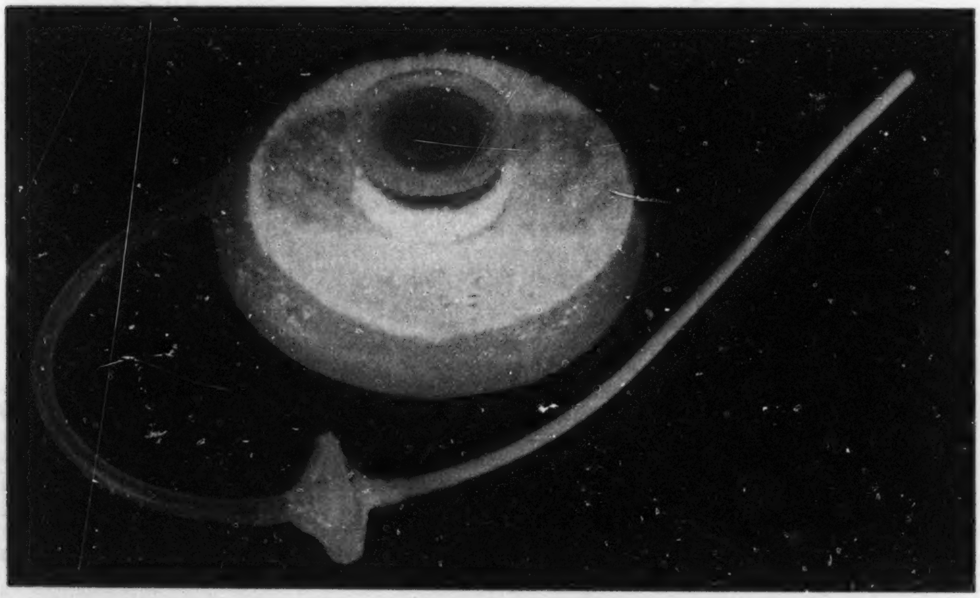
A new system for delivering insulin directly into the peritoneal cavity has eliminated recurrent and sometimes life-threatening infections caused by administering the drug through intravenous catheters, an unusual route of administration reserved for diabetics who do not respond to conventional injections of the drug.

"Subcutaneous tissue in these patients suddenly and without any apparent reason begins to degrade and inactivate insulin. Intravenous catheters may then be implanted," says Dr. R. Philip Eaton, professor of medicine at the University of New Mexico (UNM) in Albuquerque, "but after several weeks or even days, the catheters sometimes become infected or thrombose and obstruct the flow of blood."

To solve the problem Drs. Eaton and David Schade, associate professor of medicine at UNM, developed a system that delivers insulin directly to the peritoneum, where the portal vein absorbs and carries the drug to its target organ, the liver. The system, which has been approved by the UNM Institutional Review Board and is implanted under Food and Drug Administration guidelines, consists of a dacron and silicon port implanted near the umbilicus and sealed by the body's own skin. Insulin is injected through the port and into a tube that leads to the peritoneum. The system does not intrude into the circulatory system, it is completely sealed from external bacteria, and the insulin is self-sterilizing; for these reasons, the risk of thrombosis is eliminated, and the chance of infection is greatly reduced.

Since December 1981 the new system has been implanted in six patients whose recent medical history showed frequent and life-threatening infections or thromboses due to the use of intravenous (IV) catheters. Prior to implant, the patients averaged 14 days per month in the hospital. None was able to attend work or school. "They were in desperate situations," Dr. Eaton says. "One patient, over a 2-year period, had a total of 27 IV catheters implanted and then removed because of complications."

The peritoneal delivery system is still functioning well in five of the six patients, allowing each to resume normal activities free from the high risk of complications. The first system implanted was removed by attending physicians in Minnesota because of a suspected infection surrounding the injection port. "We think that multiple injections into the port, which in this patient was located below the skin, caused local pain and irritation," Dr. Eaton says. "Because of that problem we changed the port's location from an internal to an external site."



Peritoneal insulin delivery system: *buying time for the diabetic who doesn't respond to conventional injections of insulin.*

Self-injection of insulin into the peritoneal delivery system: *painless access to the target organ, the liver.*

The injection port is circular and about one-half inch in diameter. Its dacron cuff is sewn to the skin surface. The healing process causes skin to grow into the cuff and bond with it. "So now the patient injects directly into the port and not through the skin," Dr. Schade says. "Therefore we think infection is very unlikely. We've since implanted this modified system in three patients, and they're all doing well." The original system is also operating well in two of the first three patients.

The system was developed for a very specific purpose—to treat patients whose subcutaneous tissue degrades and inactivates insulin. The syndrome may appear at any stage in a diabetic's life, and it may disappear just as suddenly. "The syndrome is very poorly understood. It's a phenomenon that does not appear to be permanent," Dr. Eaton says. Generally it lasts for only a few months. Those receiving the drug delivery system at UNM, however, are exceptions. All have had the problem for at least 2 years and show no signs that it will end soon.

Time is one factor in deciding whether to implant the system; another factor is how seriously the disorder affects the diabetic. A candidate referred by an Oklahoma physician has been affected for 1½ years and has been in the hospital for the past 3 months.

"If I knew that within a few weeks this patient would respond to insulin shots, I would not consider implanting the system. But I don't know that, and there's no way to find out," Dr. Eaton says. "When we implant the system, what we're really doing is buying time, alleviating life-threatening situations that occur with intravenous treatment, and freeing patients from recurrent and sometimes lengthy hospital stays that prevent them from pursuing a normal social or economic livelihood."

This was the case for the most recent implant patient—an 8-year-old girl from Washington State who had experienced repeated life-threatening infections from IV catheters. About a dozen other patients, referred by physicians across the United States, are now waiting to receive the system.

Patients travel to the UNM General Clinical Research Center (GCRC) in Albuquerque for surgery that requires less than 45 minutes to complete. The technique, which was developed by former UNM surgeon Dr. Jay Gregory, requires only local anesthesia. Dr. William Sterling of the UNM surgery department now performs the operation. Dr. Sterling first inserts one end of a coiled silicon rubber tube into the peritoneal cavity, where it floats freely. The other end is attached to an injection port machined from polypropylene and fitted with a trilayer silicon rubber septum. He then sews the port into the skin so that the rubber septum protrudes through an incision near the umbilicus. Over a period of several days, skin grows into the dacron cuff that encircles the septum, forming a natural seal against infection.

The patient remains at the GCRC for about 2 weeks to ensure that the incision heals properly and that the device is correctly positioned and functioning. That period of time is also needed to train the patient to use the system. Usually the patient's physician visits the GCRC a few days after surgery to see how the system is implanted and how it works.

From the patient's viewpoint, there is little difference in administering insulin by subcutaneous injection or through the peritoneal device. "Diabetics have been self-injecting insulin ever since the treatment was available. The major difference between conventional treatment and peritoneal delivery is that the insulin is carried directly to the liver. Previously it could only be given subcutaneously, where it was absorbed by the veins and routed through the heart, through the arteries, and finally to the liver. By then most of the insulin was broken down."

The patient must be alert for signs of infection, but infection is not a serious concern. By providing a natural seal for the system, the risk of bacteria infecting the peritoneal cavity is minimal. Even insulin contaminated by bacteria poses little danger. "Prepared according to the U.S. Pharmacopeia formulation, the insulin contains antibacterial agents, so that bacteria contaminating the insulin will die within 24 hours," Dr. Eaton says. "The key to allowing these ingredients to work is to have a reservoir and a long tube, so that the insulin injected today doesn't reach the body until at least 24 hours later. The reservoir and tube we use hold the insulin for twice the required time."

Absorption by the portal vein of old insulin pushed into the peritoneal cavity by new injections is metered by the clinical formulation of the drug itself. The type of insulin recommended by the UNM researchers is formulated to be absorbed within 6 hours.

Dr. Eaton recommends that diabetics self-inject an appropriate amount of insulin into the peritoneal system periodically and especially at meals and before sleep, when they need a large supply of insulin. This schedule simulates the normal pattern of insulin secretion by the pancreas. It also manually approximates the action of insulin pumps, which are being developed by various research groups around the world. (See *Research Resources Reporter*, May 1979.) These pumps administer a continuous and varying flow of insulin tailored to the activities of the individual.

Five years ago, when Dr. Eaton and his colleagues began work on an implantable insulin pump at the Albuquerque GCRC, peritoneal delivery of insulin was only a concept, unproved and untried. Basic research by the two physicians determined that insulin injected into the peritoneal cavity is absorbed significantly, though not totally, by the portal vein, which transports it directly to the liver, as with insulin secreted by the pancreas.

"Almost in parallel with the scientific evolution of the concept for peritoneal delivery of insulin and the development of a pump, we found this unforeseen way of manually administering insulin with the peritoneal port," Dr. Eaton says. "It's an ideal example of how the GCRC program is having a very real impact on patient care."

Meanwhile, the semiautomatic implantable pump developed at UNM has been used successfully in several patients. The pump can be programmed to release more or less insulin depending on daily schedules. It releases this insulin in a constant flow, which Drs. Eaton and Schade believe is better for the patient than sudden subcutaneous injections. The pump system also includes a memory of doses given each day.

"The advantage that the manual peritoneal system and the pump system have over conventional treatment is the route of administration—to the liver through the peritoneal cavity," Dr. Schade says. "The advantage unique to the pump is that it essentially runs itself, requiring only instructions on what to do. The pump is much more sophisticated than the manual system."

Although the manual system was developed primarily to circumvent a specific problem that is usually transient, even patients who eventually outgrow the syndrome may decide to continue using the peritoneal port system, which guarantees direct and painless administration of the insulin. "Having direct access to the liver *could* have tremendous long-term impact on the control of diabetes and its complications," he says, adding cautiously that the benefits will take years to document. "This is the problem with new therapies for chronic diseases. The data are years away. That's why we turned our attention first to patients who really need an alternative therapy. Patients who can gain definite benefits immediately."

Additional reading:

Schade, D. S., Eaton, R. P., Warhol, R. M., Gregory, J. A., and Doberneck, R. C., Subcutaneous peritoneal access device for type I diabetic patients nonresponsive to subcutaneous insulin. *Diabetes* 31:470–473, 1982.

Development and testing of the peritoneal port for insulin delivery was supported by the American Diabetes Association, the General Clinical Research Centers (GCRC) Program of the NIH Division of Research Resources, the Juvenile Diabetes Foundation, and the Kroc Foundation. The UNM General Clinical Research Center is funded by the GCRC Program.

Hyperactivity Problems Identified in Adults

Stimulants may reduce or eliminate a variety of personality problems associated with adult hyperactivity, according to researchers at the University of Utah in Salt Lake City. "About two-thirds of our patients benefited from stimulant medication," says Dr. David Wood, a psychiatrist at the University of Utah.

Since the 1930's stimulants have been prescribed to treat children with hyperactivity, but little is known about the efficacy of stimulants in adults with the disorder. The need for such research is reflected by the severity of the problem. According to researchers at the University of Utah General Clinical Research Center (GCRC), approximately two-thirds of all hyperactive children may continue to have psychological problems as adults. These patients may be misdiagnosed as having anxiety, mood disorders, or simply being prone to antisocial behavior.

In 7 years of studying adult hyperactivity, Dr. Wood and other researchers at the Utah GCRC have tested about 200 patients. A major focus has been to evaluate the ability of stimulants to relieve symptoms of adult hyperactivity, which include motor hyperactivity, attention deficits, moodiness, and explosive temper.

The Utah researchers tested the stimulant pemoline in a 6-week trial involving 64 hyperactive adults. The re-

Drs. Frederick Reimherr (left) and David Wood, hyperactivity researchers at the University of Utah: *two-thirds of all hyperactive children may continue to have psychological problems as adults.*

searchers found that patients who were extremely hyperactive as children responded significantly better to pemoline than did other patients. Dr. Frederick Reimherr, a member of the Utah research team, says that patients who were not overly hyperactive as children often developed drug side effects including headache, nausea, cramps, anorexia, and insomnia. Four patients discontinued the drug therapy because they could not tolerate these effects.

Some patients responded immediately. Others responded only after side effects became tolerable and the dosage was increased. Some responded favorably only after receiving twice as much of the drug as other patients.

"Patients who responded well reported that, after treatment, concentration became easier, their attention wandered less, and they were able to complete tasks," says Dr. Paul Wender of the Utah team. "There was a decrease in motor activity and talkativeness. Thinking was slowed. These individuals found themselves listening to their spouses for the first time. Outbursts of temper were less severe and less common. Subjects reported that they felt less burdened and that they were able to endure everyday stresses and strains."

The effects seemed different from those reported by normal people who abuse amphetamines and experience exhilaration, loquaciousness, excitement, and restlessness. "The improvement was similar to that seen in children with hyperactivity who show a good drug response," says Dr. Wender. "Although one man rated his response as slightly improved, his wife was extremely enthusiastic about this improvement and said that years of marital difficulties had been arrested; she was no longer considering divorce."

This recent study of pemoline was developed from a trial led by Dr. Wood in 1975, in which the effects of the euphoric stimulant methylphenidate hydrochloride and the stimulant pemoline were compared. Fifteen subjects in that study were given methylphenidate. Two weeks later 11 subjects were switched to pemoline or tricyclic antidepressants. If the patient responded well to pemoline, it was continued. If he or she responded well to methylphenidate but failed to react to pemoline, the patient was put back on methylphenidate. Those who did not respond favorably to either drug were then given antidepressants. At the end of the study, which lasted 12 months, 8 of the 11 subjects were judged by the researchers as having an overall good response to drug treatment. Some patients responded to more than one drug. Fifty-three percent of the patients responded favorably to methylphenidate, 33 percent improved with pemoline, and 8 percent transiently improved when receiving tricyclic antidepressants. The rest did not respond to any drugs.

"Those responding to the drugs exhibited an increase in concentration, energy, and calmness and a decrease in irritability and moodiness. But none of the patients reported an increase in 'happiness' after methylphenidate treatment," says Dr. Wood. The researchers found that behavior improvement of patients could be maintained for months on low dosages of methylphenidate.

Such a failure to develop tolerance is characteristic of the response of hyperactive children, unlike that in stimulant abusers, who must rapidly increase dosage to obtain the desired effects. Patients who responded to pemoline treatment improved markedly more than those who responded to methylphenidate. "These patients became less anxious, less irritable, and less angry," he says. "Moods became stable and the subjects became less impulsive."

The researchers believe that patients who responded to stimulants may be hyperactive due to a persistent biological abnormality. Dr. Wender says that research by other investigators showed a higher incidence of alcoholism and personality defects among parents whose natural children were hyperactive than among parents who had adopted hyperactive children.

Accounts given by patients studied by the Utah researchers indicate that approximately one-third of their parents and one-fifth of their siblings were alcoholics or abused other drugs. Of the patients' 34 children, 41 percent had been independently diagnosed by other psychiatrists as being hyperactive. These observations indicate that hyperactivity may be inherited and that in some instances hyperactivity is a childhood forerunner of difficulties in adult life, says Dr. Wender.

Other evidence supporting the theory that some types of the hyperactive syndrome are hereditary stems from research into the effects of stimulants. Because stimulants act on the metabolism of nerve cells in the brain, Dr. Wender says, hyperactive individuals who respond to these drugs may do so because the drugs correct defects involving certain neurochemical messengers. This may explain why some hyperactive children outgrow the disorder: their neurotransmitter levels may naturally become normal, according to Dr. Wender.

He says there is an increasing amount of evidence suggesting that hyperactivity is associated with a deficiency of dopamine, a neurotransmitter that controls emotional response and complex movements. Stimulants are more effective than tricyclic antidepressants in controlling hyperactivity in children, he points out, and stimulants increase dopaminergic activity, while antidepressants increase noradrenergic and serotonergic neurotransmitter activity in the brain.

Drs. Wender, Wood, and Reimherr now are looking at cerebrospinal fluid for possible abnormalities that might reflect deranged brain metabolism. They have obtained cerebrospinal fluids and 24-hour urine samples from hyperactive adults to measure dopamine and other neurotransmitter levels. Dr. Wender says that preliminary results may indicate a deficiency in dopamine metabolism.

The researchers are conducting further tests that may determine the origin of hyperactivity. They also hope to find improved methods of drug treatment by testing several compounds that previously have not been used to treat hyperactivity. These new compounds include phenylalanine, a naturally occuring amino acid, and deprenyl, a monoamine oxidase inhibitor.

Additional reading:

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Research into hyperactivity at the Utah GCRC is supported by the General Clinical Research Centers Program of the NIH Division of Research Resources, the National Institute of Mental Health, and Abbott Laboratories.

Vitamin E May Prevent Development of Tumors in Mice

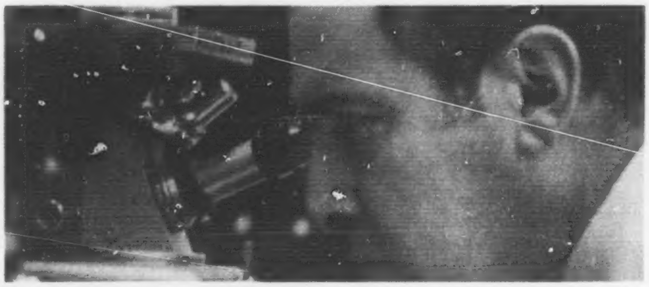
Vitamin E at controlled levels in the diet may inhibit the proliferation of tumorigenic cells, according to a recent research study with mice at the Boston University School of Medicine. Although previous research in mice has indicated that vitamin E can prevent carcinogen-induced mutation of a normal cell into a tumor cell, this study demonstrates that vitamin E can inhibit sarcoma cells from developing into tumors.

If similar results can be demonstrated in humans, then certain doses of vitamin E may prevent, not only initial tumor growth, according to researchers, but also the metastasis of cells from the original tumor. It is hoped that this might prevent the recurrence of tumors from the small number of tumor cells left following surgery for breast cancer or other solid-tumor cancers, according to Dr. Laurence Corwin, professor of microbiology at Boston University School of Medicine.

The purpose of Dr. Corwin's study with Dr. Michael Kurek, currently an applications specialist at the Millipore Corporation in Massachusetts, was to determine how vitamin E and dietary fat interact to affect tumor development. Several research studies have indicated that level of fat and type of fat appear to be important factors in tumor progression and that, with the exception of melanoma cells, most cancer cells grow best in animals fed diets high in polyunsaturated fats (PUFA's), which suppress the immune system.

The quantity of vitamin E needed to successfully prevent development of certain mouse sarcoma cells into tumors is dependent on the amount of PUFA's in the diet and on the number of cancer cells present, according to Dr. Corwin. He and Dr. Kurek demonstrated vitamin E protection against tumor production by varying the levels of dietary PUFA's and of vitamin E. They fed one group of mice 0.5 gram of vitamin E per kilogram of a diet high in PUFA's and a control group, a diet high in PUFA's but without the vitamin E supplement. They injected the mice with derivative sarcoma cells that they had previously demonstrated grew best in PUFA's. Mice fed the vitamin E supplement diet developed significantly fewer tumors than mice fed the unsupplemented diet. Similar results were produced with mice injected with sarcoma cells from the parent cell line, which the investigators had previously demonstrated grew best in saturated fats. Mice fed 0.5 gram of vitamin E per kilogram of a diet low in PUFA's developed significantly fewer tumors than mice fed an unsupplemented diet low in PUFA's.

"For vitamin E to be effective in preventing the growth and development of tumorigenic cells into tumors, dosage is important," says Dr. Corwin. "In one study we varied the vitamin E dosage in mice. We observed a bell-shaped response curve that indicated an optimum preventive dose of vitamin E that works, given a certain level and type of fat and number of tumor cells. We also found that if we gave the mice too much vitamin E, the vitamin lost its ability to protect against tumor development. Megadoses appear to decrease the tumor-preventing action of vitamin E. Our in vitro treatment of tumor cells with excessive levels of vitamin E corroborates this conclusion."



Dr. Laurence Corwin: *inhibiting the growth of mouse tumor cells with vitamin E.*

"It is difficult to extrapolate this data to humans by making dietary recommendations of vitamin E intake," adds Dr. Corwin. "I believe that vitamin E would work in humans, but I can't say what the proper dose would be. A preventive dose may very well correspond with the current recommended daily allowance of 12 to 15 international units. I say this because we recently found that vitamin E protects against tumor production in mice even at 0.05 gram per kilogram of diet, which is the normal vitamin E requirement in the diet of a mouse. Thus, popping vitamin E pills is certainly not what I'm recommending."

Vitamin E inhibits tumor production by strengthening the antigenicity of tumor cells, according to Dr. Corwin. Antigens are substances that evoke a specific immune response. Most tumor cells have weak antigens, which may not trigger the immune system, allowing them to develop into tumors. By strengthening the antigens of these cells, vitamin E exposes them to recognition and attack by the immune system. Increased antigenicity sparks a strong enough immune response to prevent the tumor cells from proliferating into tumors, according to Dr. Corwin.

"We demonstrated this in vitro," asserts Dr. Corwin. "We found that sarcoma cells grown in a medium containing vitamin E after a few generations provoked a much stronger immune response in vitro than sarcoma cells that had been cultivated in the same medium without vitamin E. Our research focus now is to identify what specific part of the immune system is responding to this increased antigenicity."

Additional reading:

1. Kurek, M. P., and Corwin, L. M., Vitamin E protection against tumor formation by transplanted murine sarcoma cells. *Nutrition and Cancer*. In press.
2. Corwin, L. M., Varshavsky-Rose, F., and Broitman, S. A., Effect of dietary fats on tumorigenicity of two sarcoma cell lines. *Cancer Research* 39:4350–4355, 1979.
3. Tengerdy, R. P., Heinzerling, R. H., and Nockels, C. F., Effect of vitamin E on the immune response of hypoxic and normal chickens. *Infection and Immunology* 5:987, 1972.
4. Stephenson, J. R., and Aaronson, S. A., Antigenic properties of murine sarcoma virus-transformed BALB/3T3 nonproducer cells. *The Journal of Experimental Medicine* 135:503–514, 1972.
5. Shamberger, R. J., Baughman, F. F., et al., Carcinogen-induced chromosomal breakage decreased by antioxidants. *Proceedings of the National Academy of Sciences* 80:1461–1463, 1973.

Research on vitamin E protection against tumorigenesis is supported by the National Cancer Institute, the U.S. Department of Agriculture, and the Biomedical Research Support Grant Program of the NIH Division of Research Resources.

Research Resources Reporter
November 1982

Information Exchange

Research Highlights

Abnormal DNA fragments have been identified in DNA/anti-DNA antibody immune complexes isolated from systemic lupus erythematosus (SLE) patients by researchers at Harvard Medical School in Boston, Massachusetts. The discovery may explain the origin of antibodies against the patient's own tissues in this disease.

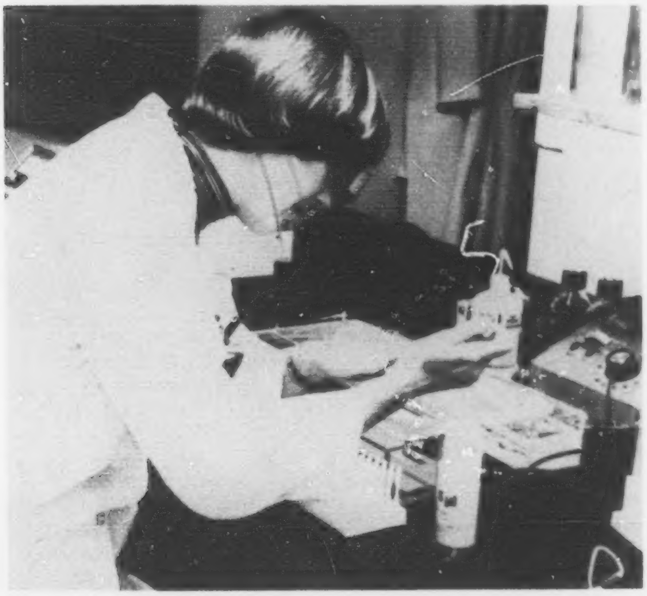
Two classes of abnormal DNA were identified in the complexes isolated from serum and plasma of SLE patients. In both, the guanine-cytosine (G-C) content differed significantly from that in normal DNA. The small abnormal DNA fragment (mw 25,000) had an average G-C content equal to 55 percent of the total of bases, that is, the purine and pyrimidine content. In the large fragment (mw 100,000) the G-C content was 45 percent of the total of bases. Normal human DNA has a G-C content of 38 to 40 percent of the base total. "Such unusually high G-C content can alter DNA structure so that it may become a target for antinuclear antibody production," says Dr. Hiroshi Sano, principal investigator. He also points out that DNA with high levels of G-C sometimes forms unusual structures such as triple-stranded forms, left-hand DNA, and bent DNA. Presumably, these bizarre forms, significantly different from normal cellular DNA, could trigger the immune system to form anti-DNA antibodies.

Dr. Sano explains that the G-C-rich fragments in SLE could be produced in two different ways. First, chromosomal DNA could be degraded randomly into fragments, and some of these with high-level G-C content might stimulate the formation of antibodies. Secondly, chromosomal DNA might be prone to break at G-C-rich regions, resulting in release of a high concentration of G-C-rich fragments into the blood, which might induce antibody formation. DNA hybridization studies indicated that both DNA fragments isolated from the immune complexes originated from human nuclear DNA.

SLE is one of the autoimmune diseases in which the body manufactures antibodies against itself. Some of the symptoms of SLE have been postulated to result from immune complexes—antigen-antibody-complement—that are deposited in various tissues. Autoantibodies may also combine directly with the patient's tissues. White blood cells migrate to these areas and phagocytize the complexes, but in the process they spill enzymes that cause inflammation and tissue injury. Joints, skin, blood vessels, the nervous system, kidneys, and often other organs of the body become chronically inflamed and injured. The disease is not uncommon, and studies indicate that more than 95 percent of the patients have antinuclear antibodies.

The body's immune system normally recognizes its own tissues as "self" and does not attack them. If cellular constituents such as nucleoprotein become sufficiently altered, however, the immune system may react to destroy them, as it does to a foreign invader of the body. The cause of these tissue alterations in autoimmune disease is not known, although it has been postulated that in certain cases they may result from viral infection.

Recently Drs. Sano and Chikao Morimoto of Harvard Medical School, Boston, have isolated DNA fragments with high G-C content from lymphocytes of SLE patients. These fragments were somewhat larger than those isolated from the immune complexes, but the G-C content was approximately the same. The researchers believe that these fragments could also serve as antigens to induce formation of autoantibodies that could react to the patient's own tissues.



Technician readies DNA samples for electrophoresis: ***looking for abnormal DNA fragments that may induce autoimmune response in patients with SLE.***

Additional reading:

1. Sano, H., and Morimoto, C., DNA isolated from DNA/anti-DNA antibody immune complexes in systemic lupus erythematosus is rich in guanine-cytosine content. *Journal of Immunology* 128: 1341–1345, 1982.
2. Williams, R. C., and Bankhurst, A. D., Clinical immunologic studies in systemic lupus erythematosus. *Arthritis and Rheumatism* 21 (suppl.):202, 1978.
3. Koffler, D., Agnello, V., Thoburn, R., Kunkel, H. G., Systemic lupus erythematosus: prototype of immune complex nephritis in man. *Journal of Experimental Medicine* 134:169, 1971.
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Research into studies of abnormal DNA in systemic lupus erythematosus is supported in part by the Biomedical Research Support Grant Program of the NIH Division of Research Resources and by the Arthritis Foundation.

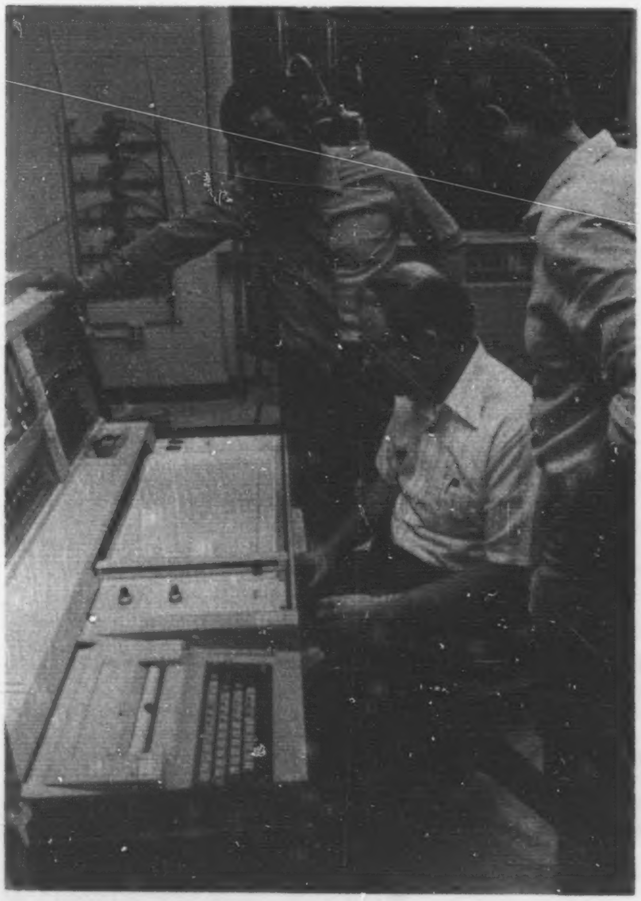
Research Service

Multinuclei NMR Spectroscopy Resource

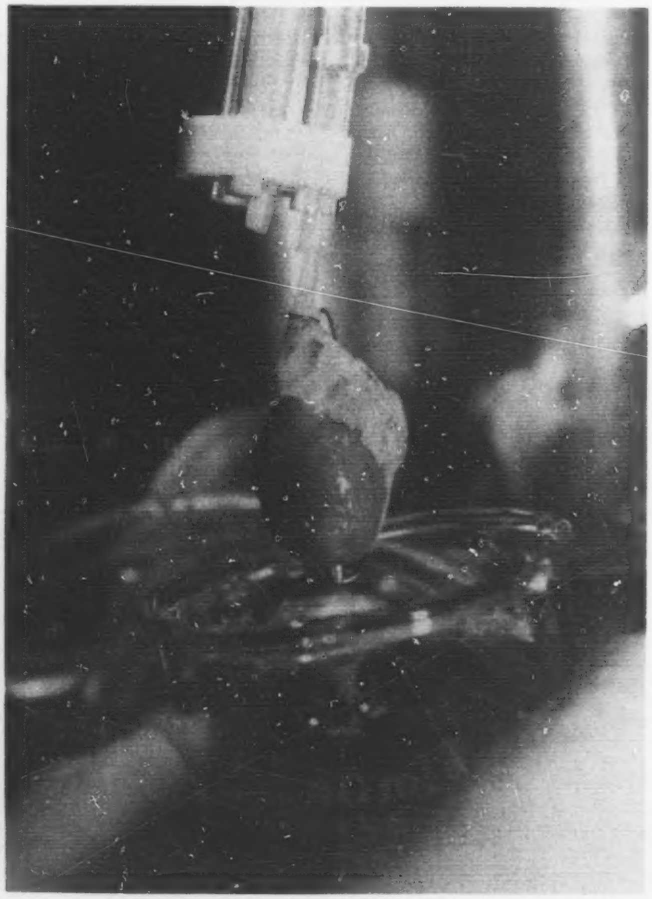
A resource that combines multinuclei nuclear magnetic resonance (NMR) spectroscopy with data processing capabilities is available at Syracuse University, New York. The resource is supported by the Biotechnology Resources Program of the NIH Division of Research Resources.

With NMR spectroscopy, scientists study the structure and interaction of molecules. When atoms are placed in a strong, uniform magnetic field and are exposed to a radio signal tuned to specific frequencies, the nuclei absorb and retransmit the radio signal. Resulting data indicate the sample's molecular structure. A multinuclei NMR spectrometer enables scientists to study molecules containing more than one type of atomic nucleus.

The Resource for Multi-Nuclei NMR and Data Processing is intended primarily for scientists whose studies require lengthy periods of data acquisition and highly sensitive NMR spectroscopy of ^{13}carbon, ^{15}nitrogen, and ^{31}phosphorus nuclei. Researchers can communicate directly with the Syracuse University resource computer by telephone. Through NMR/SPECNET, scientists can obtain data from experiments in progress or send raw data to the resource computer and receive real time, digital, or graphic output. The resource soon will sponsor semiannual workshops and training programs and will make available videotape instructions demonstrating the



Dr. George Levy (left), director of the Resource for Multi-Nuclei NMR and Data Processing with staff members Drs. Paul Marchetti and A. Ejchart (right): *providing data acquisition for lengthy NMR studies involving ^{13}carbon, ^{15}nitrogen, and ^{31}phosphorus.*



Rat heart studied at Syracuse University: *model for metabolic studies aimed at protecting the human heart during open heart surgery.*

NMR spectrometer's operation and the computer capabilities.

According to Dr. George C. Levy, director of the resource, the facility is the first in the country to combine NMR spectroscopy with spectroscopic data processing. The laboratory contains two high-field spectrometers and two computers. Primary instrumentation includes a Bruker WM-360 wide-bore 8.5 Telsa multinuclear NMR spectrometer. Broadband probes and solenoid coil probes enable the study of small to moderate-sized samples that are from less than 0.3 ml to 1.5 ml. Other resource instrumentation includes a 3.5 Telsa wide-bore multinuclei NMR spectrometer and a 5.8 Telsa narrow-bore multinuclei NMR spectrometer.

The data processing equipment includes a Data General MV-8000 32-bit minicomputer running a graphics-implemented spectral data reduction software system for one- and two-dimensional NMR spectroscopy. Two high-speed color graphics terminals are interfaced directly with the resource computer. These terminals are based on Lexidata 3400 image processors and have a direct memory access (DMA) interface to the 32-bit computer. Features of the spectral data processing system ORACLE, which is copyrighted, include full floating point computation and extended capabilities for quantification of spectral lines, cross-correlation spectroscopy, spin relaxation experiments, and general spectrum conditioning including sophisticated baseline correction algorithms. A multilayered architecture with automatic operations by default and several levels of manual overrides makes ORACLE both powerful and easy to use. Spooled output includes versatile formats of digital plots, which are fully annotated with text.

Graphics terminals and plotters in remote laboratories are supported through standard telephone connections. Scientists in remote laboratories can interact with resource personnel through the computer for the purpose of monitoring experiments in progress at the resource, thereby eliminating the need for travel to the resource. Full data sets can be transmitted to or received from remote laboratories, and interfaces to commercial spectrometer data formats are available.

The laboratory houses a biochemical preparations facility and office space for visiting scientists and coworkers. In addition, the two major software systems developed at the resource, ORACLE and ORACLE 2D, which is also copyrighted, are being disseminated to other laboratories.

For further information, contact:

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Resource for Multi-Nuclei NMR
and Data Processing
Department of Chemistry
Syracuse University
Syracuse, New York 13210

Meetings

November 14–17, 1982

Title: *3rd Annual Meeting of the Society of Environmental Toxicology and Chemistry*
Location: *Arlington, Virginia*
Contact: *Richard E. Tucker*
P.O. Box 352
Rockville, Maryland
20850
Telephone (301)
468-6704

November 16–18, 1982

Title: *20th Annual International Medical and Laboratory Instrumentation Congress and Exhibition*
Location: Washington, D.C.
Contact: *John L. Wolf*
AIMLI
11310 Palisades Court
Kensington, Maryland 20895
Telephone (301) 946-6464

**November 29–
December 1, 1982**

Title: *Immune Networks*
Location: *New York, New York*
Contact: *Conference Department*
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021
Telephone (212) 838-0230

December 2–3, 1982

Title: *16th Annual Meeting of the American College of Chemosurgery*
Location: *New Orleans, Louisiana*
Contact: *Cheryl Nordstedt*
Convention Office
1567 Maple Avenue
Evanston, Illinois 60201
Telephone (312) 869-3954

December 2–4, 1982

Title: *2nd International Symposium on Medical Virology*
Location: *Anaheim, California*
Contact: *Luis M. de La Maza, M.D.*
Department of Pathology
University of California
Irvine Medical Center
101 City Drive
Orange, California 92668
Telephone (714) 634-6868

December 2–4, 1982

Title: *The Flexibility of Endoscopy: Update December 1982*
Location: *New Orleans, Louisiana*
Contact: *Dr. Myron Lewis*
Course Director
The American Society for Gastrointestinal Endoscopy
13 Elm Street
Manchester, Massachusetts
01944
Telephone (617) 927-8330

December 3–5, 1982

Title: *International Society and Federation of Cardiology*
Location: *Caracas, Venezuela*
Contact: *E. G. J. Olsen, M.D.*
Department of Histopathology
National Heart Hospital
Westmoreland Street
London W1M 8BA, England

December 4–7, 1982

Title: *24th Annual Meeting of the American Society of Hematology*
Location: *Washington, D.C.*
Contact: *Registration Supervisor*
Charles B. Slack, Inc.
6900 Grove Road
Thorofare, New Jersey 08086
Telephone (609) 848-1000

December 4–9, 1982

Title: *41st American Academy of Dermatology Annual Meeting*
Location: *New Orleans, Louisiana*
Contact: *Cheryl K. Nordstedt*
1567 Maple Avenue
Evanston, Illinois 60201
Telephone (312) 869-3954

December 11–12, 1982

Title: *12th Annual Clinical Dialysis and Transplant Forum*
Location: *Chicago, Illinois*
Contact: *D. Chianchiano*
National Kidney Foundation
2 Park Avenue
New York, New York 10016
Telephone (212) 889-2210

**December 19, 1982–
January 1, 1983**

Title: *8th Annual Bicentennial Medical-Dental Seminar*
Location: *Vail, Colorado*
Contact: *F. B. Glenn, M.D.*
7741 Southwest 62nd Avenue
South Miami, Florida 33143
Telephone (305) 661-6208

January 10–12, 1983

Title: *3rd Annual Meeting of the American Society for Laser Medicine and Surgery*
Location: *New Orleans, Louisiana*
Contact: *E. H. Drake, M.D.*
425 Pine Ridge Boulevard
Suite 203
Wausau, Wisconsin
54401
Telephone (715) 845-9282

February 28–March 4, 1983

Title: *International Academy of Pathology, U.S.-Canadian Division*
Location: *Atlanta, Georgia*
Contact: *N. Kaufman, M.D.*
1003 Chafee Avenue
Augusta, Georgia 30904
Telephone (404) 724-2973

March 2–5, 1983

Title: *4th Annual Meeting of the Society of Behavioral Medicine*
Location: *Baltimore, Maryland*
Contact: *Thomas J. Coates, Ph.D.*
Society of Behavioral Medicine
P.O. Box 8530
University Station
Knoxville, Tennessee
37996
Telephone (615) 974-5164

March 14–16, 1983

Title: *5th Charles River International Symposium on Laboratory Animals, "Biomedical Research: Importance of Laboratory Animal Genetics, Health, and Environment"*
Location: *Heidelberg, Germany*
Contact: *Symposium Chairman*
Charles River Foundation
P.O. Box 430
Wilmington, Massachusetts
01887
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Research Resources Reporter

December 1982, Volume VI, No. 12

U.S. DEPARTMENT OF HEALTH
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Serving Animal Resources,
Biotechnology Resources,
General Clinical Research Centers,
Minority Biomedical Research Support,
and Biomedical Research Support

This Issue:

Primates in Research
Endotoxic Shock Therapy
Detecting Carriers of DMD
Genbank

By
Greg Freiherr

Laser Surgery Mends Nerves

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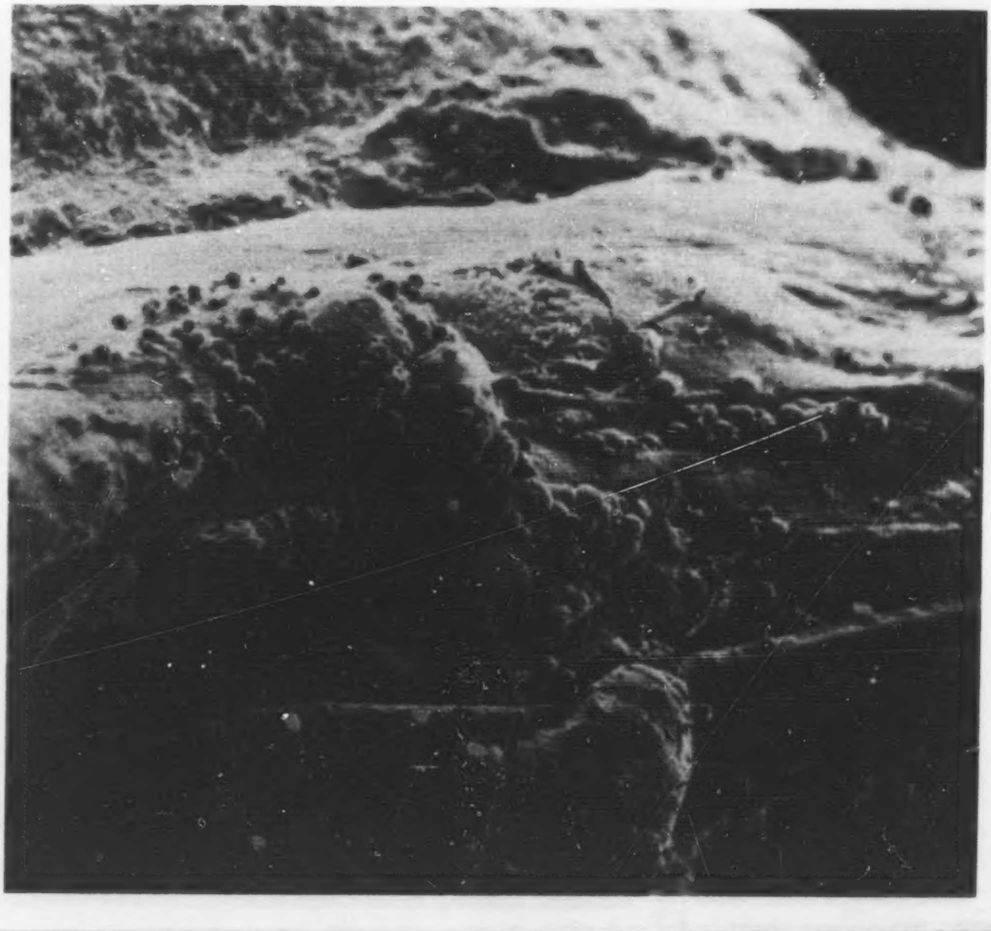
A laser beam coagulates red blood cells encircling the ends of a severed nerve, forming an adherent sleeve of tissue that holds the two ends together. Within this sleeve, new nerve fibers from the ends grow together.

Whether laser repair of the damaged nerve will produce better results than suturing the nerve ends together is not known. The technique has just recently been applied to humans, and conclusions on the return of the nerve function cannot yet be drawn. Only white rats, monkeys, and baboons have been completely evaluated.

The animals were studied at the University of Washington (UW) in Seattle, where Dr. Edward E. Almquist, a hand surgeon who developed the laser procedure, is a clinical professor in the orthopedics department of the medical school. Tests on the nonhuman primates were conducted at the UW Regional Primate Research Center in Seattle.



Severed rat nerve 8 weeks after repair with a laser: ***coagulated red blood cells (nodules in center) still adhere to the healed junction.***

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LASER *continued*

Dr. Edward E. Almquist, Seattle hand surgeon and developer of the laser procedure for repairing nerves: *"If the (nerve) fibers can be closely aligned there is a much greater probability that significant sensation will return."*

Success in the animal tests was judged mostly on the appearance of the repair sites several months after surgery. Nerves were examined with scanning transmission electron microscopy and light microscopy. "The external surface of the repair sites could often barely be distinguished from the adjacent nerve surface or from nerves that had not been severed," Dr. Almquist says. The quantitative evaluation of sensory return, however, could not be determined. "Injury to one nerve and the consequent loss of sensation affect coordination and dexterity but not necessarily gross motion," he says.

These questions will be decided through ongoing clinical studies at the Seattle clinic of Dr. Almquist and his colleagues in the Seattle Hand Surgery Group. They are applying the laser technique to people with nerve damage in the upper extremities.

Glass, sheet metal, and power tools inflict the majority of these injuries. According to a 1977 survey by the National Center for Health Statistics, more than 20,000 injuries to nerves in the arm, wrist, and hand occur annually. "Damage of this type can stop the means of employment for someone who requires a great deal of dexterity—someone like a watch repairman or a surgeon," Dr. Almquist says.

The injury is at least an inconvenience—a nagging, frustrating one that makes simple actions difficult. Writing, buttoning shirts, or just tying shoes can be a problem. The hope that surgical reconnection of the nerve might restore at least some sensation has been drawing people for years to the clinic of the Seattle Hand Surgery Group.

Before the laser was introduced, nerves were usually reconnected by suturing the severed ends together. This method is still generally used. It works, but it has some drawbacks. The fibers from each nerve end branch unpredictably, a phenomenon demonstrated in numerous animal experiments. Even the most advanced microsurgery, with sutures so small that individual nerve branches are tied together, cannot prevent these branches from sprouting outside the intended junction sites.

As a result, most nerve fibers do not reconnect with sister fibers from the other part of the nerve. Those that do reconnect usually do so incorrectly. "The fibers that normally provide feeling to the thumb might attach to fibers that serve the ring finger," Dr. Almquist says.

Children typically improve more than adults when severed nerves are sewn back together. The reason, according to Dr. Almquist, is that the child's brain is more adaptable. "Even though the nerve ends don't match very well, the brain is eventually able to make sense of the information it receives," he says.

There is another drawback to sewing nerves together. "The suture goes through the body of the nerve, so it does some damage," Dr. Almquist says.

To increase the number of nerve fibers that correctly reconnect and to reduce damage to the nerve, a variety of sleeves made of fibrinogen, silicon, glass, veins, or hypoallergenic collagen were developed and tested. None proved clinically practical until Dr. Almquist and his colleagues fashioned a natural sleeve from

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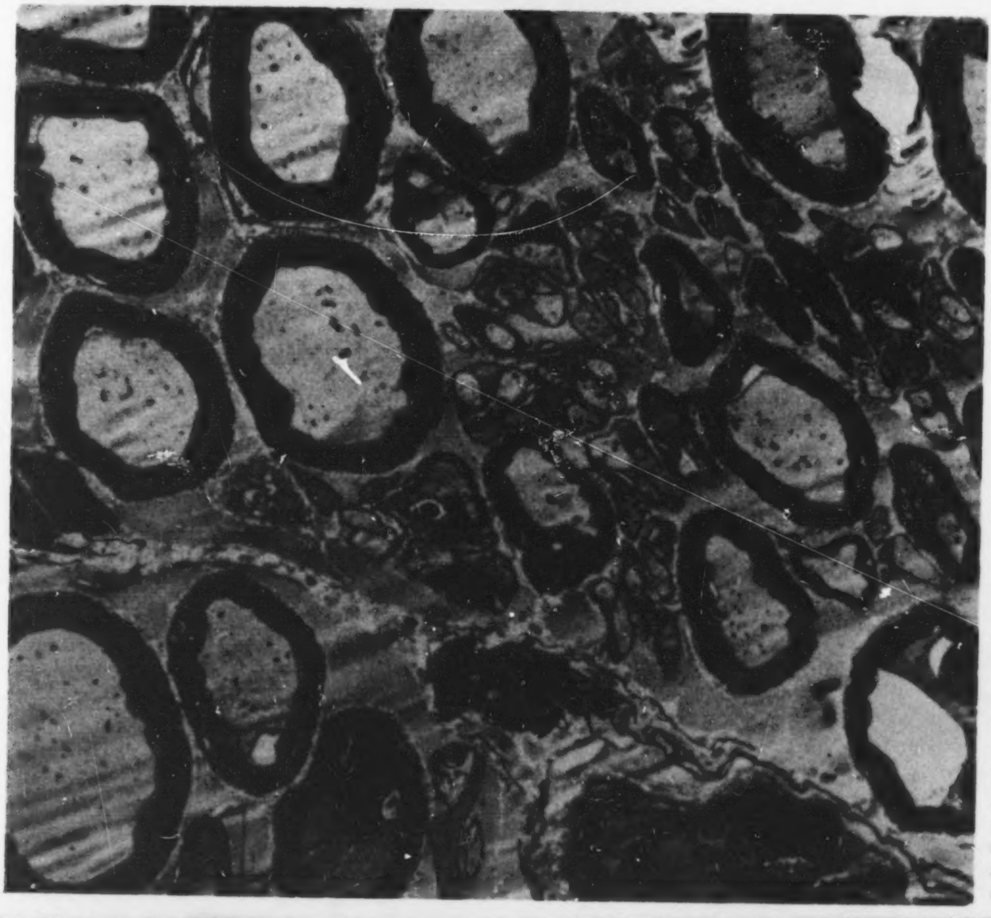
Two baboons at the Primate Field Station near Spokane, Washington: *aiding in the repair of nerves with laser light.*



Nerve fascicles (circles): *bundles of nerve fibers analogous to the wires inside an electrical cable.*

coagulated red blood cells. The sleeve directs the growing fibers to each other. It also holds nerve fibers together without sutures.

"Previously we didn't bother matching the nerve fibers because the branches would grow out of alignment and the effort would be wasted. But if the fibers can be closely aligned there is a much greater probability that significant sensation will return," he says.

When splicing a severed nerve with the laser, Dr. Almquist matches from each nerve end bundles of nerve fibers called fascicles. The process is analogous to matching and splicing the wires inside an electrical cable. Matching nerve fibers, however, is immensely more difficult than the task facing an electrician. Each fascicle measures about 0.1 millimeter in diameter and contains about 10,000 fibers.

The laser surgeon examines the nerve through a binocular microscope suspended over the patient. He looks on the surface of the fascicles for characteristic ring patterns; for example, a wide ring followed by two thin ones. Corresponding nerve bundles are held together with a forceps. Blood is applied around the junction site and the area is flashed with the laser.

The surgical system consists of an argon ion laser fitted with an optical fiber, a shutter, timer, and foot switch. Depressing the foot switch opens the shutter for a preset time. The laser beam travels through an optical fiber consisting of fired quartz. Its length is 4.5 meters; its core diameter is 400 microns, which is equivalent to the diameter of the graphite in a pencil.

The laser delivers only 0.75 watts of power, and its burst of light lasts just half a second. The blue-green light is absorbed by red-colored materials—the red blood cells. It is reflected by white materials—the nerves. As planned, red blood cells applied over

LASER *continued*

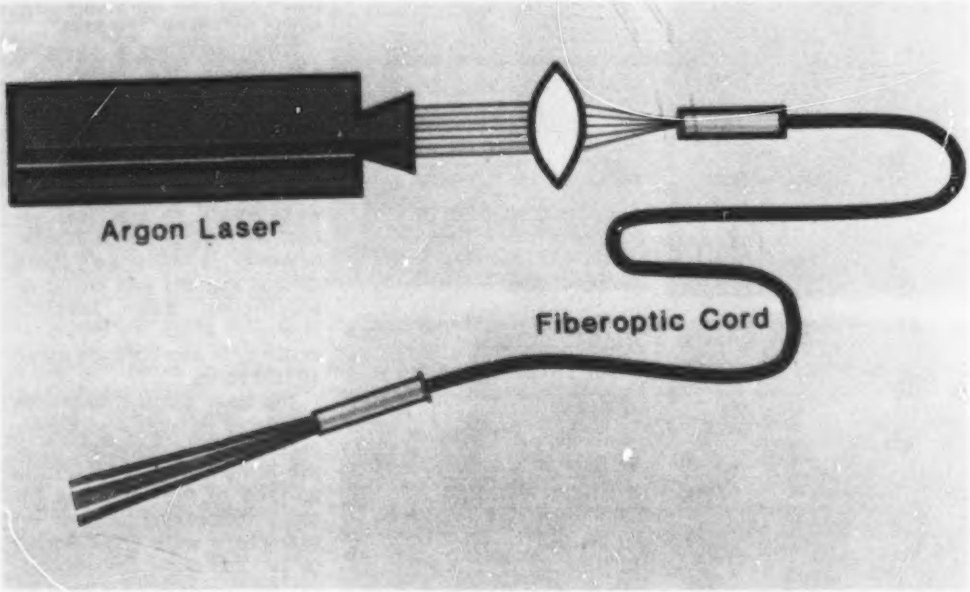
the nerve absorb the energy from the beam and form a sleeve of coagulated tissue. The white nerve fibers reflect the laser energy.

Although the laser light itself does not affect the nerve, heat from the coagulation of red blood cells might do some damage. Such a small quantity of heat is generated, however, that a means of measuring it has not yet been devised.

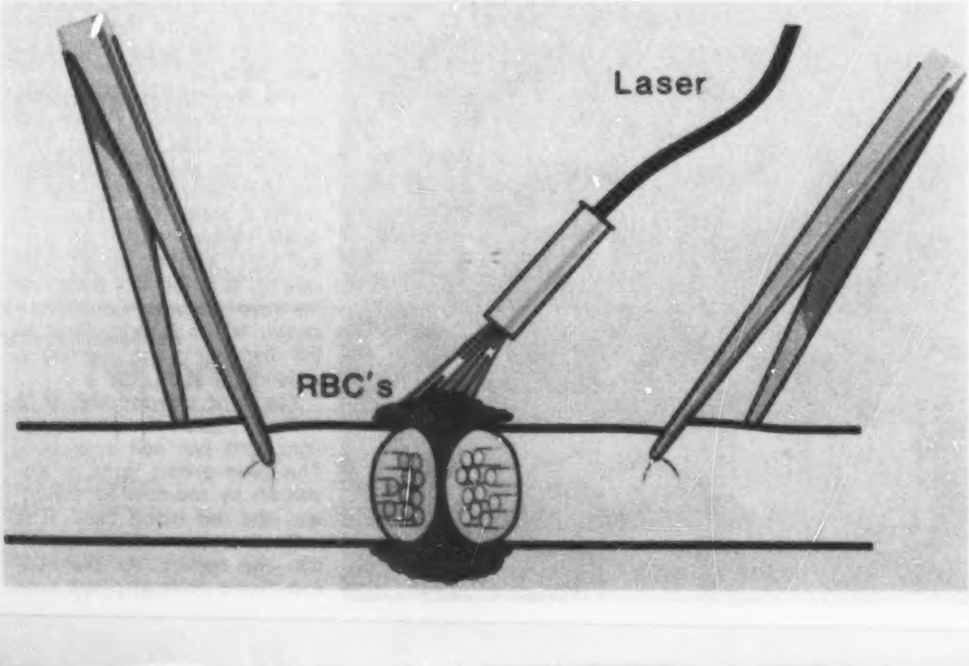
Feasibility of the technique was proved on rats in 1976, using an argon laser focused through a microscope. A prism and mirror assembly directed the beam. Although nerve repair appeared to be technically possible, the system was awkward and could not be used successfully on even a major nerve. When the microscope was replaced by an optic fiber system, developed by Dr. David Auth of the UW Seattle electrical engineering department, the technique became practical.

Tests on 32 rats confirmed that the procedure allowed severed nerves to heal. Electron microscopy and standard light microscopy of nerves harvested at various times following surgery showed how the healing progressed.

At first, adherent round globules formed from the red blood cells hold the nerve ends together. Within several days, collagen fibrils intermesh in the sleeve and replace the globules, and nerve fibers begin to sprout from the nerve ends. The sleeve of coagulated red blood cells constrains the



Surgical laser system



Laser light coagulates red blood cells (RBC's) encircling the severed nerve ends.

sprouts to the repair site. Within 3 months after surgery the repair site is nearly indistinguishable from the rest of the nerve surface as seen by electron and light microscopy.

In the rat, the laser-repaired nerve produced a return of muscle function. "A word of caution about interpreting results in rats: previous studies of other repair procedures including simple suturing have shown similar repair and a return of function," Dr. Almquist says.

The truly encouraging news came in the next phase of the research—the testing of 10 nonhuman primates at the UW Regional Primate Research Center in Seattle. Each animal was anesthetized. The left median nerve, which serves muscles of the forearm, thumb, elbow, and many joints of the hand, was cut and dissected microscopically into 3 or 4 fascicles. Blood mixed with heparin was applied over the fascicles as they were held together with forceps. A flash of laser light coagulated the blood, creating a sleeve over the two nerve ends.

The ulnar nerve, which serves many of the same areas as the median nerve, was used as a control. It was exposed, covered with the blood-heparin mixture, and flashed with the laser. Areas of the median and ulnar nerves exposed to the laser were retrieved 4 to 6 weeks after surgery.

As in the rats, primate nerve repair sites were nearly indistinguishable from other surface areas. Nerve fibers were channeled and joined together. Few fibers escaped from the sleeve. Dr. Almquist judged the alignment and density of the regrown nerve ends in the primate to be "very satisfactory." There was no excessive scarring. The ulnar nerve, which was not severed, showed no damage due to exposure to laser light or to heat resulting from the coagulation of blood cells.

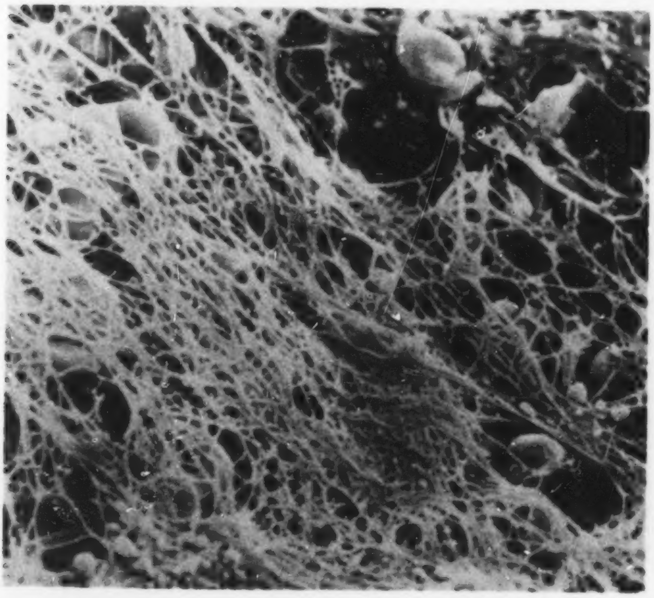
But the ultimate question is whether the repair of nerves with a laser will return more sensation to fingers and hands than does conventional surgery in which the nerve ends are sewn together. The clinical studies now going on should prove the answer. Evaluating the return of sensation, however, will be subjective.

"You can't say that a patient has a 50 percent return of feeling, because there is no good way to quantitate feeling. What's normal feeling? What's half normal feeling? We don't know," Dr. Almquist says. "We will have to depend on comparisons by the patient of sensation after surgery to sensation before and after the accident."

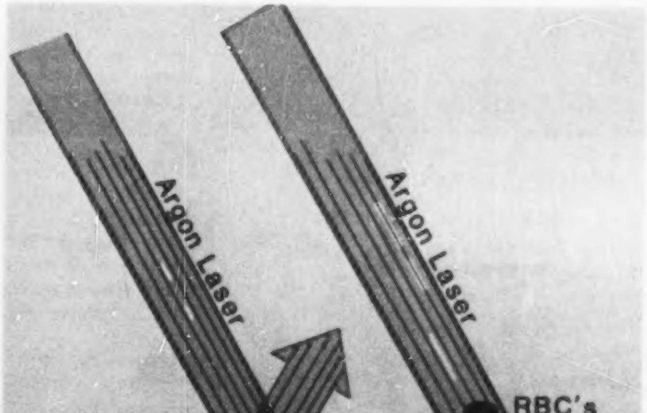
Preliminary results will soon be available. Sensation begins to return about 3 to 4 months after surgery, and it continues to increase for another year. "But we will have to wait years for the final answer—until enough time has elapsed and enough patients have undergone the treatment," says Dr. Almquist, "that we can confidently render a judgment."

The University of Washington Regional Primate Research Center in Seattle is supported by the Animal Resources Program of the NIH Division of Research Resources.

Coagulated tissue surrounding the severed nerve ends: ***a natural sleeve that holds the ends in place and guides growing nerve fibers to their targets.***



Red blood cells (RBC's) absorb the blue-green laser light, but white nerve fibers reflect it: ***applying the laser's power while protecting the nerve.***



Primates in Biomedical Study: Resources To Be Conserved

At the Regional Primate Research Centers (PRC's), the animal—not the facility—is the resource. Living animals are the proving grounds of ideas that cannot be tested on people. They must be in excellent health to ensure that test data are valid. When the animals die, tissues and specimens are conserved for study. About 13,000 primates, representing 44 species, are now housed in the Regional PRC's, which are supported by the Animal Resources Program of the NIH Division of Research Resources (DRR). The large number and variety of animals are distinctive of PRC's.

Core breeding programs supply about 1,800 animals per year—about 70 percent of the subjects needed for center research projects. The University of Washington PRC maintains a breeding colony near Spokane, Washington, and a small research station in Borneo, Indonesia. The breeding colony, with some 1,500 primates, supplies animals to the PRC laboratories in Seattle, where studies in neurophysiology, cardiovascular physiology, developmental biology, metabolism, and genetics are conducted. Some research is also done at the breeding station, particularly long-term studies of aging. The research station in Borneo serves as an outpost for the study of primates in the wild, where information useful in care, handling, and breeding of the animals can be obtained.

In an interview with Greg Freiherr of the *Research Resources Reporter*, the director of the University of Washington PRC, Dr. Orville Smith, discussed the role of animals in biomedical research, ethical considerations in their use, and alternatives to animal studies.

Dr. Orville Smith, director of the University of Washington Regional Primate Research Center in Seattle: ***"People shouldn't be the first living organisms to take an experimental drug or vaccine."***

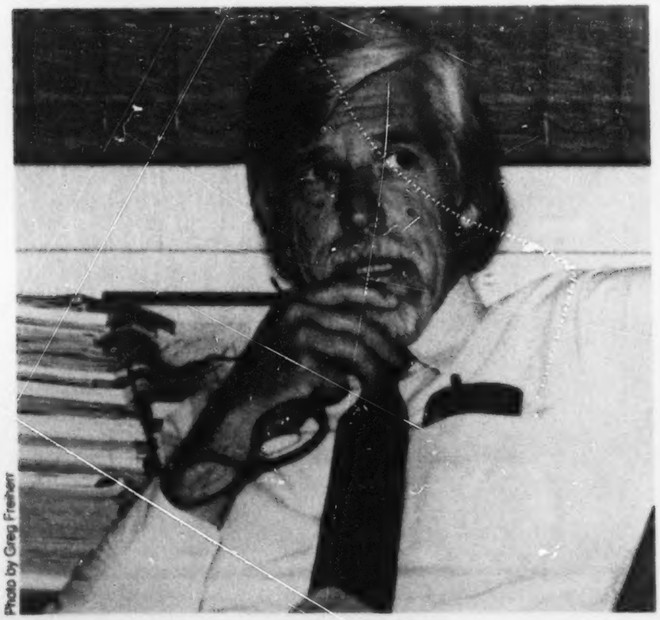


Photo by Greg Freiherr

Why use nonhuman primates in research?

In many cases they provide research opportunities that no other kind of experimental animal can provide. Humans and nonhuman primates have the same essential characteristics with regard to anatomy and physiology. Our biochemical makeup is similar. From a biological standpoint, the probability is much higher that research carried out on a nonhuman primate is going to be directly applicable to human beings than research using some other animal.

What types of studies are done best on primates?

The visual systems of the monkey and the human are almost indistinguishable, whereas the visual systems of the cat, dog, guinea pig, and rabbit are all very different from man's. The basic organization of the human and nonhuman primate brains is essentially the same: humans have a central sulcus, a motor cortex, and a sensory cortex in the same places as does the nonhuman primate. Because of the similarities in brain structure there is reason to believe that the neural mechanisms underlying the animal's behavior are similar to the human's. Another example is the cardiovascular system. The baboon is a better model of blood flow to the kidney during heavy exercise than is the dog. One reason may be that the dog is a running animal with a large spleen, which provides a reservior of blood. The dog acquires extra oxygen-carrying capacity in the bloodstream during exercise because the spleen constricts, releasing extra blood. The baboon and the human don't have this capacity.

Then animals are chosen according to the goals of the specific study?

Exactly. It depends on the mechanism we want to study. We'd much prefer that animals other than the primate be used whenever possible. I really believe use of the primate should be restricted to studies in which it's obvious that the primate must be the model.

This is mostly because primates are expensive, isn't it?

No. Not entirely. Economics enter into the decision, but the major factor is conservation—the general principle of using our animals efficiently and wisely.

Isn't it also true that the use of animals phylogenetically closer to man generates greater concern among animal rights activists than the use of animals farther down the evolutionary ladder?

I think that's undoubtedly a true statement, but there is much concern today about the welfare of all animals in research. It's an issue that is being hotly debated. Historically, and even today, the public's greatest concern has been with animals that are also kept as pets, such as cats and dogs.

The central point in this debate is whether animals are really needed to conduct research. Are they really needed?

At this time they are absolutely indispensable. People shouldn't be the first living organisms to take an experimental drug or vaccine. And even after the tests are completed, we need animals to ensure the product's quality. Some years ago, quality control failed at a company that makes polio vaccine, and a number of children contracted the disease from the vaccine. If no animals were ever used for testing, our polio vaccination program could be worse than the natural occurrence of the disease. The need for animals in research is simply one aspect of the critical and continuous need for animals in our present system of health care. You either settle for unnecessary human suffering and death or you use animals.

The human-animal link: *"Our best protection against cruelty to animals is the people who handle the animals."*

Do you agree that alternatives such as in vitro testing should be used to reduce the use of animals?

A lot of preliminary research is already being done in vitro. That's great. The more we can do in cell culture, the better we like it. But there are limitations. We can go only so far. With in vitro methods, we aren't going to learn a single thing about how the integrated brain operates, for example. We need a functioning, intact brain—an organ with billions of nerve cells interacting with each other.

In the areas suited to alternative methods, could these alternatives be used more extensively?

In your question you presume that the alternative technology is available and that maybe there isn't the interest to apply it. That's not the case. Cell culture is not that old. Investigators are and have been applying it wherever it can be applied. Work involving recombinant DNA is being done now because the tools are available. We're developing alternatives by our own volition, and we're applying them when the technology fits the problem. What's exciting is that the technology is becoming available, and researchers are now starting to use it.

But isn't there some resistance in the biomedical community to using the alternatives that are now available and applicable?

I don't think so. While the amount and quality of biomedical research has been increasing in the last 15 years, the number of animals used has decreased by 35 percent. Most of us, I think the great majority, would prefer to use alternatives, but they simply don't exist right now for many major avenues of research. Existing alternatives are being used. Ironically, some of the ostensible alternatives beg the question of whether it is morally right to use animals. One example is the use of tissue cultures. The cells for tissue cultures have to come from animals, and even after they're extracted, the cells are alive. So where do you make the cutoff?

Isn't the cutoff determined by either of two attitudes? One relates to ethics: Should animals be used? The other is concerned with the kind of experiments using animals—the cruelty question.

Those who cite the ethics of animal research state that no form of animal life should be injured for any reason. Some adhere very conscientiously to their code of ethics. They are vegetarians. They do not wear leather shoes, and, similarly, they are very careful not to wear clothes made of leather or fur. I don't knock that. I admire ethical principles. If a person believes in them and acts accordingly, I respect that. But I just don't see how any person who has a real ethical sensibility can say that his or her children, friends, or parents should suffer or die rather than take the life of an animal.

INTERVIEW *continued*

Is the antivivisection movement composed mostly of absolutists?

There are all varieties of people in the movement. Some are absolutists. But many want to work with the scientists to improve the daily existence of the animal in the laboratory. They're not saying: "Stop your research." They're saying: "Let's make sure that these animals are cared for in the best possible fashion, that they experience the least distress possible, and that they have the best facilities and the best veterinary care."

Now you're talking about people who oppose cruelty to animals. Why aren't they satisfied with existing guidelines for the care and handling of animals?

Regulations specified in the Animal Welfare Act and administered by the U.S. Department of Agriculture address the environmental conditions under which research animals are kept—cage size, lighting, ventilation. That sort of thing. Currently the major issue is whether new laws should be passed to deal with how animals are used in experiments.

Are there no existing guidelines for how the animals are used?

Certainly there are guidelines. The NIH Guide deals with some of the basic issues regarding analgesia, anesthesia, postsurgical care, and acceptable methods of euthanasia. Scientists working at the primate center have for years adhered to more detailed guidelines developed by their professional societies, including the American Physiological Society, the National Society for Medical Research, and the American Veterinary Medical Association. These guidelines are specifically intended to minimize pain that might be experienced by experimental animals. The issue now has more to do with regulations and laws. Some believe that the Congress and the Federal agencies should play a more directive role in defining, regulating, and enforcing such guidelines.

Isn't that role now being played primarily by the university?

At our primate center, we rely on a university-wide Animal Care Committee to review the protocols of all grant requests to assure that animals are used properly. It is composed of veterinarians, researchers, administrators, and a member of the lay public. On a day-to-day basis, our veterinarians and their staffs monitor the condition of all animals. They will stop any ongoing experiment that they believe is not adhering to the principles of good care and ethical treatment. Unnecessary pain is one criterion they are concerned about.

How do you define "unnecessary pain?"

In most animal research, pain is not a common feature. Unnecessary pain is pain inflicted without a reason. We are opposed to this.

What happens if the veterinarian stops an experiment?

He ordinarily recommends alternative approaches so that the research can continue. There are often different ways to approach a research problem, and a scientist must do what he's been trained to do—what seems to be the most logical approach to answering an important question. If in pursuing that goal, the animal will have unnecessary pain, the research has to be done another way, or it has to stop.

Some researchers reportedly have discontinued their work in recent years because they didn't believe the research goals justified the pain and injury experienced by the animals. Have you or your staff ever felt that you're going beyond what should be done?

I hope that no one here would knowingly go beyond what he should do. But there is, and I think there always will be, the feeling that the conditions and treatment of animals can be better. We constantly try to improve the quality of animal care. As an example, we have found ways to drastically reduce our need for the restraint chair. Its use has made possible a tremendous amount of research that required long-term measurement of physiological or neurological functions. But we don't like the idea of an animal sitting in a chair for several weeks at a time. So, we have set out to reduce as much as possible our use of the restraint chair.

We did so by developing new techniques for surgical implants, by using a tether system for fluid lines that allows the animal freedom of movement, and by using vests that carry radio transmitters. Now we're developing a cage that turns into a temporary chair. When it's time to run tests, the animal hops onto the seat and sticks his head through the top of the cage. We give him a little piece of apple and wheel the whole cage into the study room. When the testing is done, the seat is removed and the animal is back in his cage.

Why have you made these changes?

They are ways of making the research easier on the animal. It wasn't more convenient for us to build the convertible cage, to develop better implant techniques, or to apply radio transmitters and tether systems. It would have been more convenient to just use the restraint chair. But we felt the need to improve conditions for the animals as the technology became available.

I think that serves as a good example of the concern people here at the primate center have for our animals. Our best protection against cruelty to animals is the people who handle the animals. I can't emphasize that enough. Our veterinarians have day-to-day contact with the animals. They care about how the animals feel. If the animal technicians think something is wrong, they report it to the veterinarians. Something is done about it immediately.

Nothing goes on here that isn't scrutinized by the veterinarians, the staff, and the scientists themselves. It's the only way. You can have all the regulations in the world, but if the people in charge of the animals don't care about them, the animals won't be treated properly. Our people care.

A lot is said about conserving research animals. Are animals at the Seattle primate center used in more than one experiment?

Oh, yes. One primate can help in several research programs. It's common to reuse animals. Our breeding station in Spokane makes it possible to conduct some experiments even before the animals are born—studies of fetal development, for example. After birth the animal may be studied in a developmental program, where observations contribute to our baseline knowledge of such factors as growth, blood characteristics, and social behavior. These animals are used in many projects.

Multiple use is a very important element of conservation. It's also very important scientifically. Many kinds of longitudinal data are simply not available in the human population. The only place you can get these data under controlled environmental conditions is in a primate colony. We have data on a monkey that arrived as a newborn in 1957. He's 25 years old now—the equivalent of 75 years old for a human. They don't get that old in the wild. In the wild they're either quick or they're dead. When they start to slow down, their predators have them, or other group members may attack them. So, the only way to study aging is to have a colony like ours.

If an animal participates in many projects in a lifetime, would not residual effects from previous studies compromise the data?

That depends on the studies. We have records on the animals, their clinical health, and experimental history. Whenever an animal is considered for a project, the investigator gets a readout of its history. We can choose the correct animal to use for any study.

You said earlier that, at present, animals are absolutely indispensable to conducting research. The reference to time is an important qualifier. Might animals someday be replaced by alternative methods?

The use of animals will certainly continue to be reduced. I think the greatest reduction is going to come in the area of toxicity testing with the further development of tissue culture techniques. The least reduction of animal use will be in studies that require integrated organisms. Biologists need whole animals to learn how the different organs relate and interact; for example, studies in which the nervous system has to remain integrated with other parts of the body.

But you do see an increased use of alternative tests for many areas of biomedical research?

Yes, I certainly do, and I think that's good

Do you see this change coming from external or internal forces?

It will be a combination of both. There is an increased awareness of animal welfare among the public. People are calling us to task. So, undeniably, there has been, as they say, a consciousness raising. But it's not the people outside who are making the biggest contributions. It's the people in the field who are actually making the changes. They know the problems. And, indeed, many of the solutions proposed by people outside of research are nonsense because those people don't know the problems.

One type of technology that has received a lot of attention is the computer.

That's right. Computers have achieved such an aura that people say, "Oh, you can do your experiments with the computer!" That's simply untrue. You can use the computer to analyze your data in ways that you could not before. With a computer, you may squeeze more information out of your data. But that is the full extent to which the computer can be used. You can't get anything out of a computer that you don't put into it. The computer simulation is based on data from animals, and studies of more animals are required to test the truth of predictions from the simulation. A lot of people don't recognize that.

But isn't it likely that in the future some computer simulation will be used, improved culture cell studies will be applied, and as new and better technology is developed, animals will be replaced or, at least, the number of animals needed to conduct research will be reduced?

Absolutely. Alternatives will reduce the number of animals in research. But total replacement is a long way down the line, if ever. I really doubt that we will ever be able to conduct research without any animals at all.

Microsurgery under sterile conditions at the University of Washington primate center: *adhering to detailed guidelines of analgesia, anesthesia, and postsurgical care.*

Research Focus

Therapy for Endotoxic Shock Studied in Mice

Animal experiments and data from human subjects with endotoxic shock (ES) have convinced investigators at the Medical University of South Carolina in Charleston that an inhibitor of thromboxane synthetase may improve the survival rate of patients with ES.

The drug represents a new class of pharmaceutical that inhibits synthesis of thromboxane A_2 (TXA_2), a potent vasoconstrictor and platelet aggregator, according to Dr. Perry V. Halushka, coprincipal investigator in the animal studies with Drs. James Cook and Curtis Wise. Plans are now being made to test the drug on human subjects in the late fall, says Dr. H. David Reines, the principal investigator for the proposed patient studies.

Endotoxic shock, Dr. Reines notes, is a form of septic shock caused by gram-negative bacteria. It affects the gastrointestinal tract and other parts of the body. The estimated mortality rate, investigators report, ranges from 30 percent to 70 percent, with survival depending partly on the condition of the patient before shock.

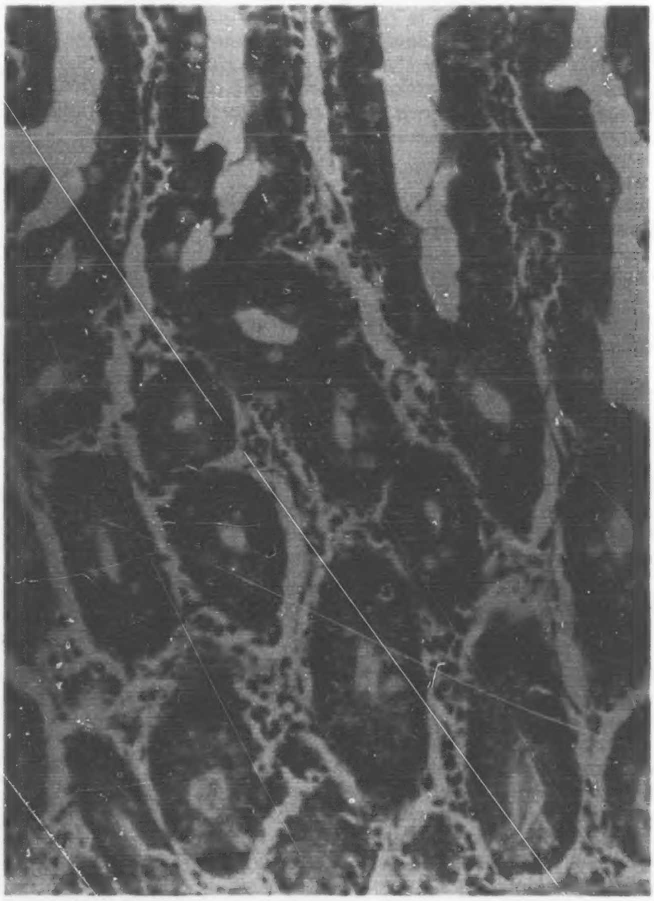
Gram-negative bacteria, which are usually present in the body without adverse effect, may infect a person who has undergone trauma or who is immunologically compromised. It is known that abdominal wounds, surgery that introduces fecal material into the peritoneal cavity, severe viral infections in children, and inflammation or obstruction of the bowel may lead to gram-negative infections. When the gram-negative bacterium dies, either naturally or because of antibiotics, it releases an endotoxin, a lipopolysaccharide, from its cell walls.

The endotoxin may cause fever, leukopenia, circulatory collapse, thrombocytopenia, disseminated intravascular coagulation (DIC), and adult respiratory distress syndrome (ARDS), which is produced by abnormally large amounts of fluid in the extracellular tissue spaces and alveoli of the lung. Animal experiments showed that ES is also associated with an increase in substances derived from arachidonic acid, a polyunsaturated fatty acid that is a component of membrane phospholipids. This last observation put researchers on the trail that led to the possible use of the thromboxane synthetase inhibitor.

"In rats with either experimental ES or a form of septic shock, there are elevations in plasma prostaglandins and thromboxanes derived from the metabolism of arachidonic acid," Dr. Halushka says. Thromboxanes are first synthesized as the highly unstable TXA_2, which may directly cause ARDS and DIC. Oxygen supply to tissues can be reduced by ARDS, and blood from the lungs can be shunted, leading to coma and death. Researchers believe DIC may lead to the massive internal bleeding that sometimes occurs in ES.

Dr. Reines and his associates found that patients who died of septic shock had elevations of plasma thromboxane levels up to 10 times greater than did patients who subsequently survived septic shock.

To inhibit TXA_2 synthesis in laboratory animals injected with endotoxin, researchers have used thromboxane synthetase inhibitors and nonsteroidal anti-inflammatory drugs such as aspirin. Both groups of drugs have significantly prolonged the survival time of rats in endotoxic shock. The source of thromboxanes is uncertain. Drs. Halushka, Cook, and Wise believe that there are multiple potential sources, including



platelets, lungs, and reticuloendothelial cells in the liver and the spleen.

Investigators hope to use a thromboxane synthesis inhibitor in clinical tests to gain a better understanding of its potential role in human endotoxic shock. "We are hoping to find a therapy for patients with DIC and ARDS," Dr. Reines says. "If we achieve our goals, then we're hoping for improved survival."

If a successful therapy is developed from these studies, it might be used to treat victims of toxic shock syndrome, an illness mainly affecting menstruating women using tampons and diaphragms. Although toxic shock syndrome is believed to be caused by gram-positive bacteria, some of its pathological mechanisms appear to be similar to those of ES, Dr. Reines says.

Whatever the results of the proposed studies, "I don't believe we will find a 'cure all' drug for ES just like that," Dr. Reines says. "Just as there is not a single cause of cancer, there is not a single mechanism in septic shock." The use of thromboxane synthetase inhibitors may be helpful to the patient only at a particular point in the development of the syndrome. Hence, the work of investigators studying other parts of the ES puzzle also holds therapeutic promise, according to Dr. Reines.

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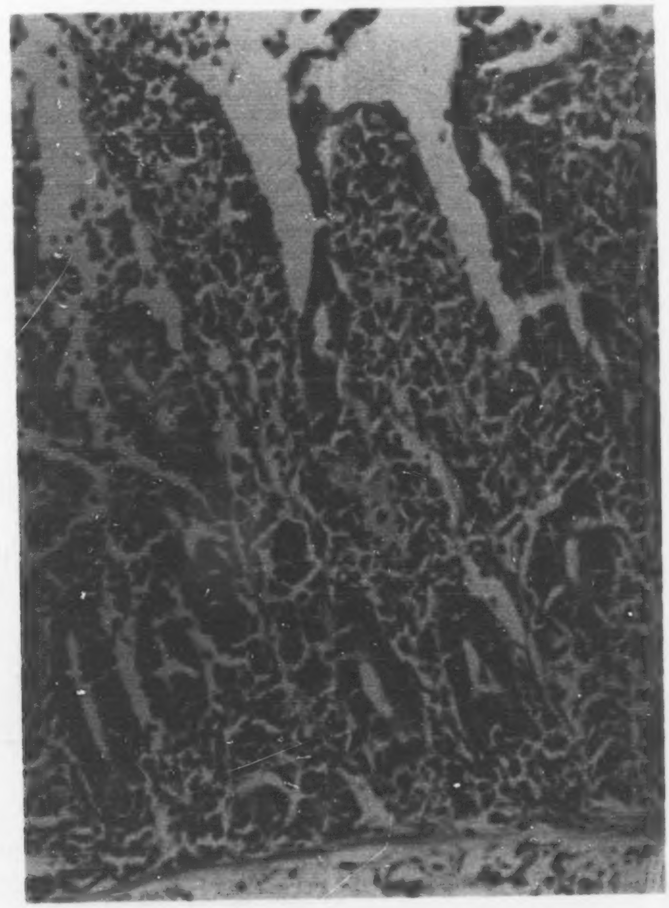
Research into endotoxic shock is supported by grants from the National Institute of General Medical Sciences, the American Heart Association, the South Carolina Heart Association, the Medical University of South Carolina Biomedical Research Appropriation, and the Biomedical Research Support Grant Program of the NIH Division of Research Resources.



Intestinal cells from rats injected with endotoxin: ***cells from a rat pretreated with thromboxane inhibitor (left) appear to be normal, whereas cells from a rat not pretreated appear to be damaged.***

Research Resources Reporter
December 1982

Information Exchange

Research Highlights

Results of a study by researchers at the Duke University Medical Center in Durham, North Carolina, may contribute significantly to more accurate detection of carriers of Duchenne muscular dystrophy (DMD), a fatal genetic disease affecting male children.

Drs. Allen D. Roses, principal investigator, and Russell J. M. Lane, of the department of medicine at Duke, found a significant variation with age in serum levels of the enzyme creatine kinase (CK) in 206 unrelated normal females ranging in age from 1 to 70. The importance of these data is underlined by the fact that the CK test is presently regarded as the best test and is the test most widely used for detection of DMD carriers. The CK level in serum is higher than normal in a large percentage of definite carriers of the disease. Detection of carriers, followed by genetic counseling, is at present the only way to control DMD.

Duchenne muscular dystrophy, named after the French physician who first described the disorder in 1861, is transmitted by mother to son. Only males develop DMD. Symptoms usually become apparent between the ages of 2 and 6. DMD is characterized by a gradual wasting away of muscle, accompanied by weakness and crippling. The disease steadily becomes worse, and children usually do not survive beyond adolescence. The cause of DMD is unknown and there is no specific therapy.

Each son of a mother who carries the Duchenne trait has a 50 percent risk of inheriting the disease, and each of her daughters has a 50 percent risk of being a carrier. Carriers very rarely have any overt signs of DMD. There are approximately 10,000 children with DMD now living in the United States, according to the Muscular Dystrophy Association, which estimates that the risk of the disease is about 1 in 4,000 live male births.

In the study conducted by Drs. Roses and Lane, CK levels were highest during the first few months of life, fell rapidly until about age 4, increased to a peak around 10 to 12 years of age, and then fell abruptly around commencement of the menstrual cycle. CK levels continued to decline slowly throughout adult life until about age 40, when there was a slight rise.

The purpose of the study, according to Dr. Roses, was to establish normal CK ranges for females at different ages—that is, age-specific CK ranges.

The fluctuation of CK values with age should be taken into account, Dr. Roses indicates, when the CK test is used for detecting DMD carriers. If the normal CK value used as the control is lower than the CK range for the age of the person being tested, normal individuals could be incorrectly classified as carriers on the basis of the CK test (false positives). On the other hand, if the control CK value used is higher than the CK range for the subject's age, carriers could be missed (false negatives). In practice, however, CK levels are incorporated with genetic data from pedigrees, for counseling purposes.

Dr. Roses states that the CK test may be most discriminating for carrier detection in children at ages 3 to 5, when the CK level in normal girls is at its lowest point.

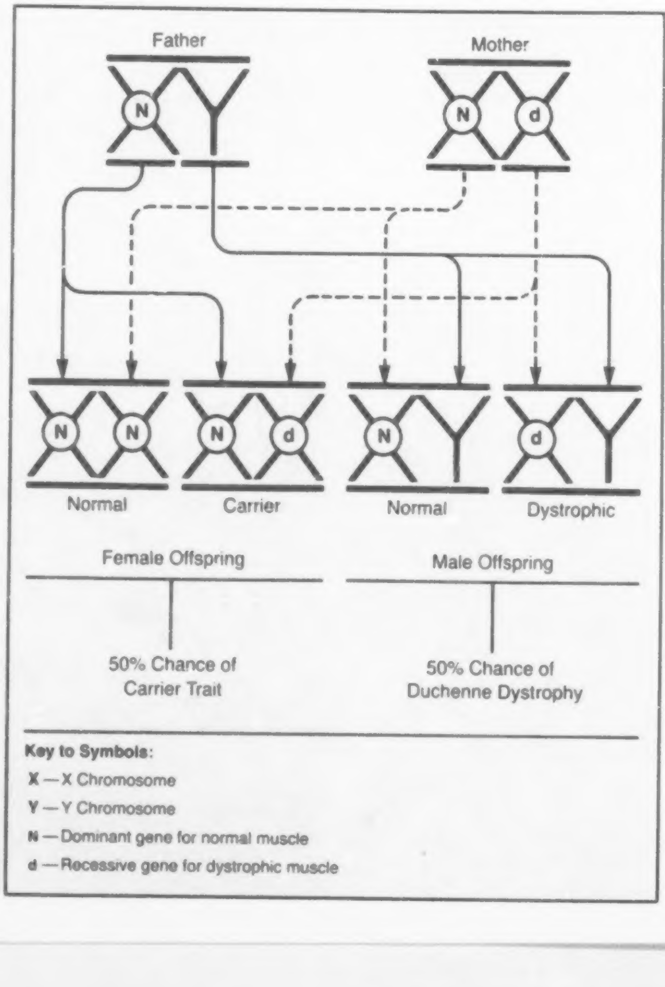
Research has indicated that CK levels are higher than normal in about 70 percent of *definite carriers* and of *probable carriers* and in about 50 percent of *possible carriers* of Duchenne muscular dystrophy.

Definite carriers are mothers who have a son with DMD and a previous case of DMD on the maternal side of the family. *Probable carriers* are mothers of two or more sons with the disease and have no other affected relatives. *Possible carriers* are mothers of a single isolated case or are a relative of an affected male.

Dr. Roses points out that the CK test is a nonspecific test since CK levels can be elevated in a number of other diseases. Some of these diseases can be ruled out by the sex of the patient, since clinical DMD occurs only in males. "Other diseases can usually be ruled out by the case history, the physical examination, and the age and sex of the patient," Dr. Roses says. "On the other hand, there are several diseases that require muscle biopsy for differential diagnosis of DMD. Biopsy is performed in all cases for which there is no family history."

Detection of DMD carriers will be more accurate when the biochemical defect in the disorder is known and potential carriers can be tested specifically for that defect, according to Dr. Roses. "Until that time, use of nonspecific measurements such as CK requires careful laboratory controls and integration of data into useful models for genetic counseling," he says.

Other procedures that have been suggested as possible DMD carrier detection tests include muscle biopsy, electrocardiography, and electro-

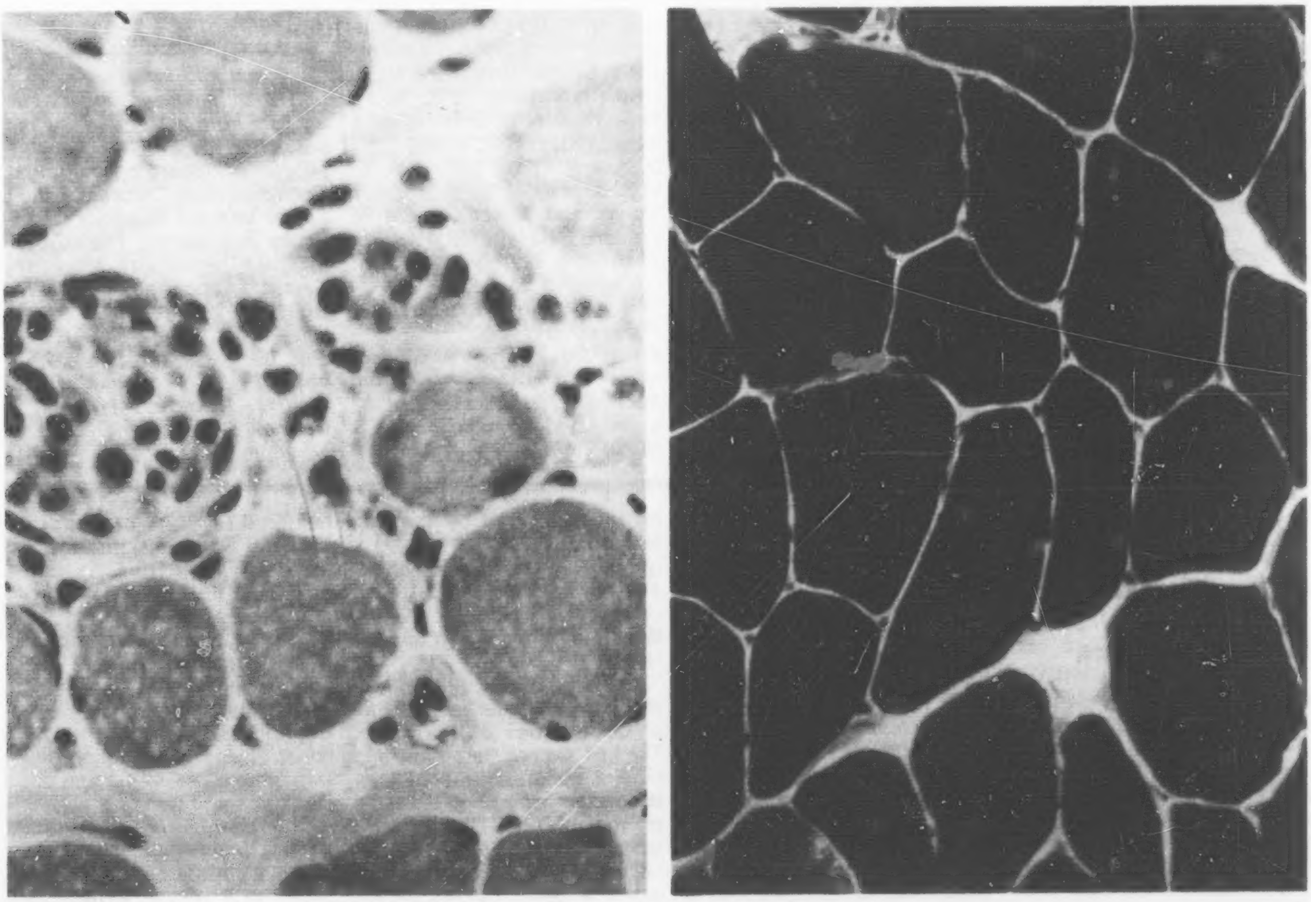

Father
Mother
N
N
d
N
N
N
d
N
d
Normal
Carrier
Normal
Dystrophic
Female Offspring
Male Offspring
50% Chance of Carrier Trait
50% Chance of Duchenne Dystrophy
Key to Symbols:
X — X Chromosome
Y — Y Chromosome
N — Dominant gene for normal muscle
d — Recessive gene for dystrophic muscle

myography. Research has demonstrated microscopic abnormalities in muscle biopsy specimens from *definite carriers*, but the procedure has been recommended only for those medical centers that have the necessary expertise in taking satisfactory biopsy specimens and have data on a large series of known carriers and normal persons as a basis for interpreting test results. *Definite carriers* with normal CK levels have been detected by electromyography, which records electrical patterns of skeletal muscle at rest and during contraction. Abnormalities in ECG have also been found in DMD carriers.

"Although the CK test is regarded as the best to date for detection of DMD carriers, it has a lot of drawbacks," Dr. Roses states. Subjective factors that affect the CK level include age, exercise, pregnancy, and individual variation from time to time in carriers. Some drugs also influence CK levels, but oral contraceptives have no significant effect. Inaccurate CK test values may also result from mishandling specimens and the use of unreliable analytical methods. To improve the value of the CK test, all of these factors must be taken into consideration.

Strenuous exercise should be avoided for 1 week prior to the test since it increases CK levels in both carriers and normal persons. Individuals vary considerably, however, in their response.

"Pregnancy almost always causes a decrease in the CK level," Dr. Roses says. Consequently, it is important to complete carrier testing and genet-



Loss of muscle cells and replacement by connective tissue in Duchenne muscular dystrophy (left) compared to normal muscle cells (right): *a crippling disorder characterized by a gradual wasting away of muscle.*

ic counseling before a pregnancy occurs.

CK levels vary considerably from time to time in some carriers. Therefore, it has been recommended that at least three tests be performed at intervals greater than 1 week. Dr. Roses emphasizes the importance of using the mean value of several samples for genetic counseling in the case of potential carriers with high or borderline CK levels.

Serum specimens should be protected from bright light and temperature fluctuations. Delay in analysis may also invalidate CK test results if the specimen is not preserved properly. In the study by Drs. Roses and Lane, to ensure accuracy, serum specimens were analyzed immediately or after storage at minus 20 degrees centigrade for a maximum of 2 weeks.

The analytical procedure used must yield both accurate and reproducible results. The importance of this is underlined by recent research that revealed marked differences in results on identical samples analyzed in different laboratories.

New research designed to find better methods of carrier detection has focused mainly on identification of the basic defect in the disease, location of the abnormal gene on the chromosome, and the abnormality in the gene itself.

Recent work suggests that there may be a generalized membrane defect in patients with DMD and that the expression of the abnormal gene is not confined to muscle. The fundamental defect could be in another organ, and the muscle abnormalities may be secondary. The actual cause of the disease beyond the genetic defect has eluded researchers so far.

Two main types of techniques are being used to locate the area on the chromosome where the genetic defect resides. One approach, mapping the DMD gene, may reveal neighboring marker systems whose inheritance may aid in the detection of DMD carriers. An example of the value of this approach is the discovery that the location of the gene for Becker muscular dystrophy is linked to that for a type of color blindness. This technique is based on the principle that each gene has a particular and well-defined location on its chromosome and that neighboring genes tend to remain together.

Dr. Roses and his colleagues at Duke are currently employing a different approach. They are using recombinant DNA techniques to search for genetic markers close to the Duchenne trait on the X chromosome. Recombinant DNA techniques may also lead to identification of the defect in the DNA of the gene itself. A probe in the area of the DMD locus might provide the basis for a direct and specific test for carriers, since practically all readily available cells of the body could be used for the test.

The Duke GCRC was used for examining some of the patients and normal controls enrolled in the study and also for obtaining some of the specimens for the CK tests, Dr. Roses says. Research by the Duke Neuromuscular Research Clinic has been centered in the GCRC since 1974.

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Research into variation of serum creatine kinase levels with age in normal females was supported by the National Institute of Neurological and Communicative Disorders and Stroke and the Muscular Dystrophy Association. The GCRC at Duke University Medical Center is supported by the NIH Division of Research Resources. Dr. Lane is now at the Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital, Newcastle upon Tyne, United Kingdom.

Research Service

DNA–RNA Data Bank

An international data bank of DNA and RNA sequences is now available. The data bank, called GenBank, enables scientists to obtain the sequences of DNA and RNA molecules comprised of approximately 750,000 bases.

Information in the data bank is being gathered, annotated, and organized by Dr. Walter Goad and his staff at the Los Alamos National Laboratory in New Mexico. A scientific research firm, Bolt, Beranek, and Newman, Inc. (BBN), Cambridge, Massachusetts, maintains the computerized data and distributes the information to subscribing institutions and scientists via magnetic tape and limited online computer access by telephone. One means of access is the PROPHET computer system, which was developed and is being maintained by the Biotechnology Resources Program of the NIH Division of Research Resources (DRR). A compendium of data in GenBank will be published annually.

An effort to develop the nucleic acid data base began at Los Alamos 3 years ago. The Los Alamos data bank and other genetic-sequence-handling tools have been available on the PROPHET system for several years. These efforts resulted in the current project, which is being funded by the National Institute of General Medical Sciences through a contract to BBN. Other agencies funding GenBank through the same contract include the National Institute of Allergy and Infectious Diseases, the National Cancer Institute, the DRR Biotechnology Resources Program, the National Science Foundation, the Department of Energy, and the Department of Defense.

The purpose of the data bank is to avoid the duplication of data collection that might occur in a rapidly expanding field such as molecular genetics. The speed at which new knowledge is gained makes it difficult for scientists to organize and interpret the information. More than 500,000 nucleic acids are sequenced each year. Understanding sequence patterns enables scientists to recognize structural features and coding relationships within DNA molecules that correspond to specific biological functions within a cell. Research in this field may ultimately help scientists understand the processes of disease as well as inheritance.

Sequences of the nucleic acids in DNA and RNA are ordered lists of four types of smaller molecules called bases. Nucleic acid molecules range in size from 100 bases to hundreds of millions of bases. The sequence of Epstein-Barr virus, which contains 170,000 bases, is expected to be published within the year.

According to Dr. Goad, the library will be brought up to date within the year. Thereafter, sequences will be collected within 3 months of their publication. The Los Alamos investigators will use information from more than 20 journals and will develop international collaborations to compile a definitive bank of DNA and RNA sequences.

Scientists who are interested in gaining access to the DNA-RNA data bank can obtain additional information by contacting:

Dr. Howard Bilofsky
GenBank
c/o Computer Systems Division
Bolt, Beranek, and Newman, Inc.
10 Moulton Street
Cambridge, Massachusetts 02238
(617) 497-2742

Meetings

Computers in Medicine Congress Announced

Computer applications in medicine will be the theme of the 2nd Annual Joint National Congress of the American Association for Medical Systems and Informatics (AAMSI) CONGRESS 83.

The conference, which will be held in San Francisco, California, from May 2 to 4, 1983, will provide a forum for physicians, nurses, health care administrators, allied health professionals, computer scientists, and bioengineers. Topics to be discussed include body imaging, X-ray and ultrasound technology, computer networks, artificial intelligence, nuclear medicine, and robotics. Ambulatory care and office practice, clinical decision-making, emergency systems, and biomedical research also will be addressed. Panel discussion will focus on clinical laboratory systems, and computer-aided medical and health education. For further information and for an application to submit papers, contact:

Dr. M. F. Collen
3451 Piedmont Avenue
Oakland, California 94611
(415) 428-6688

Conferences in Biochemistry

The annual Gordon Research Conferences, sponsored by the National Science Foundation, will be held January 10 to February 25, 1983, in Ventura, California, and January 10 to February 4, 1983, in Santa Barbara, California. The 14 conferences will be held in two cities to provide hotel accommodations for guest speakers and the approximately 1,800 invited attendees. Ten conferences will be held in Ventura, and four will be held in Santa Barbara. Each conference will last 4 to 5 days.

The conferences will update experts on the latest research developments in chemistry and related sciences. Researchers attending the conferences will discuss and analyze the significance of research developments and their underlying theories. Approaches to conducting research in these areas will be addressed.

Conferences to be held in Ventura will be on such topics as immunochemistry and immunobiology, prolactin, agricultural science, and the role of oxygen radicals in biology and medicine. Conferences to be held in Santa Barbara will be on topics including polymers, electrochemistry, and the chemistry and physics of isotopes.

For additional information, contact:

Dr. Alexander M. Cruickshank
Director
Gordon Research Conferences
University of Rhode Island
Kingston, Rhode Island 02881
Telephone (401) 783-4011

January 12–14, 1983

Title: *The Chemistry and Biology of Alpha-2 -Macroglobulin*
Location: *New York , New York*
Contact: *Conference Director*
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021
Telephone (212) 838-0230

January 17–21, 1983

Title: *Advances in Gene Technology: Molecular Genetics of Plants and Animals*
Location: *Miami Beach, Florida*
Contact: *Miami Winter Symposium*
P.O. Box 016129
Miami, Florida 33101
Telephone (305) 547-6265

January 22–29, 1983

Title: *2nd Winter Congress of the International Body Imaging Conference*
Location: *St. Moritz, Switzerland*
Contact: *Secretary*
Winter Congress
West Park Hospital
22141 Roscoe Boulevard
Department of Radiology
Canoga Park, California 91304
Telephone (213) 340-0580, ext. 280

February 12–19, 1983

Title: *2nd Winter Symposium on Hematologic Malignancies*
Location: *Snowbird, Utah*
Contact: *S. E. Jones, M.D.*
Section of Hematology and Medical Oncology
University of Arizona Cancer Center
Tucson, Arizona 85724
Telephone (602) 626-6372

February 19–24, 1983

Title: *4th International Workshop on Neurological Surgery of the Ear and Skull Base*
Location: *Sarasota, Florida*
Contact: *H. Silverstein, M.D.*
Ear Research Foundation
1921 Floyd Street
Sarasota, Florida 33579
Telephone (813) 365-0367

March 6–11, 1983

Title: *83rd Annual Meeting of the American Society for Microbiology*
Location: *New Orleans, Louisiana*
Contact: *Richard A. Bray*
1913 I Street, N.W.
Washington, D.C. 20006
Telephone (202) 833-9680

March 10–15, 1983

Title: *American Academy of Orthopedic Surgeons' 50th Anniversary Meeting*
Location: *Anaheim, California*
Contact: *C. V. Heck, M.D.*
American Academy of Orthopedic Surgeons
444 North Michigan Avenue
Suite 1500
Chicago, Illinois 60611
Telephone (312) 822-0970

March 13–17, 1983

Title: *9th Annual Meeting of the Clinical Ligand Assay Society*
Location: *Philadelphia, Pennsylvania*
Contact: *Katherine Boyle*
Wayne County General Hospital
P.O. Box 67
Wayne, Michigan 48184
Telephone (313) 722-6290

March 17–20, 1983

Title: *Annual Session of the American Association for Dental Research*
Location: *Cincinnati, Ohio*
Eloise Ullman
Contact: *AADR*
734 15th Street, N.W.
Suite 809
Washington, D.C. 20005
Telephone (202) 638-1515

March 19–23, 1983

Title: *39th Annual Meeting of the American Academy of Allergy and Immunology*
Location: *Hollywood, Florida*
Contact: *D. L. McNeil*
611 East Wells Street
Milwaukee, Wisconsin 53202
Telephone (414) 272-6071

March 20–25, 1983

Title: *185th National American Chemical Society Meeting*
Location: *Seattle, Washington*
Contact: *Paula Groves*
1155 16th Street, N.W.
Washington, D.C. 20036
Telephone (202) 872-8072

April 11–15, 1983

Title: *1st World Congress on Cardiovascular Pharmacotherapy*
Location: *Geneva, Switzerland*
Contact: *Dr. Adam Schneeweiss*
12 Avenue des Amazones
1224 Chene–Bourgeries
Geneva, Switzerland

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Research Resources Reporter

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Supplement

Serving Animal Resources,
Biotechnology Resources,
General Clinical Research Centers,
Minority Biomedical Research Support,
and Biomedical Research Support

A Cumulative Index
1982
Volume VI

ORIGINAL

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